

SDS – POLYACRYLAMIDE GEL OF OSTEOINDUCTIVELY ACTIVE
PROTEINS FROM HPLC



FIG. 1

FIGURE 2

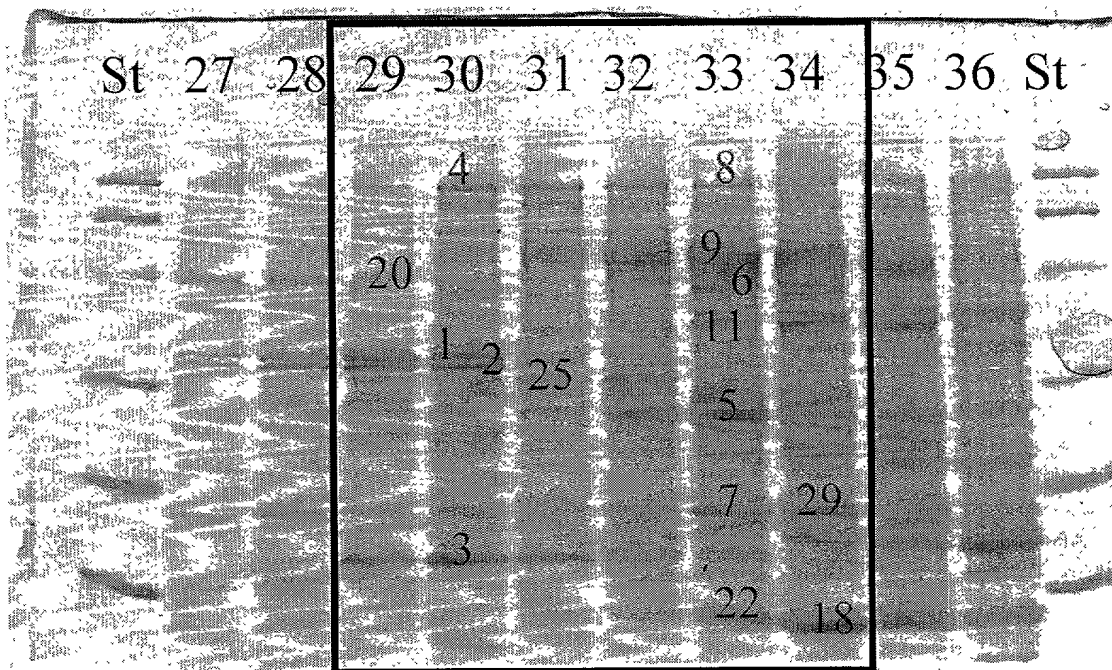
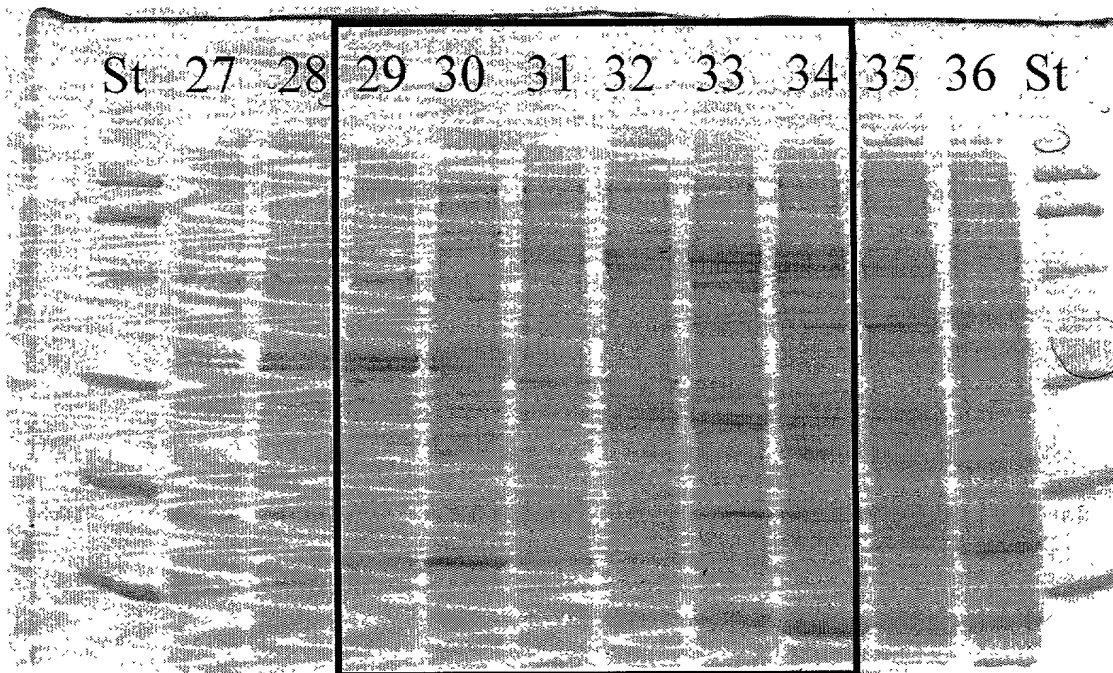
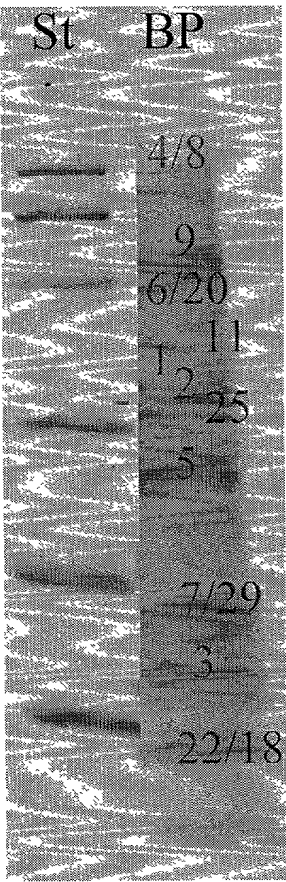


FIGURE 3



Band No.	Identity
1	Histone H1.c
2	Histone H1.c
3	Ribosomal protein RS20
4	Similar to ribosomal protein LORP
5	BMP-3
6	α 2 macroglobulin RAP and BMP-3
7	Similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	Ribosomal protein RL6 and BMP-3
18	TGF- β 2 / SPP 24
20	Factor H
22	TGF- β 2
25	BMP-3 and H1.x
29	BMP-3 and ribosomal protein RL32

FIGURE 4

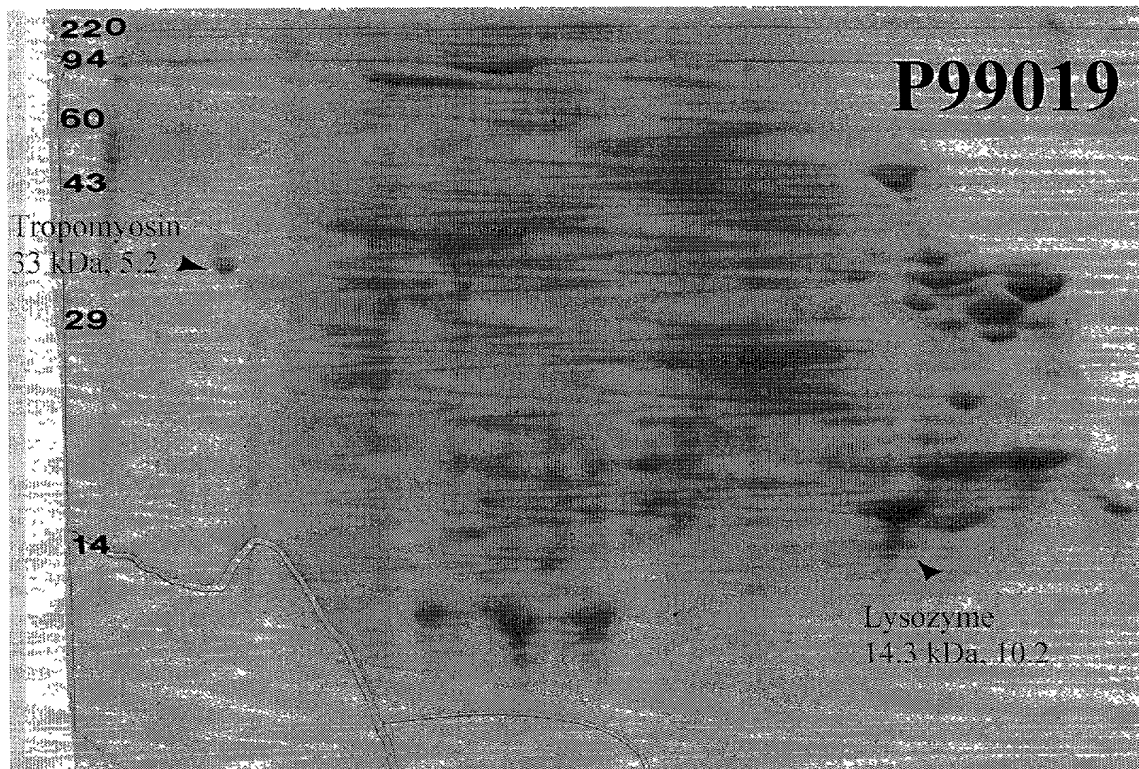


FIGURE 5

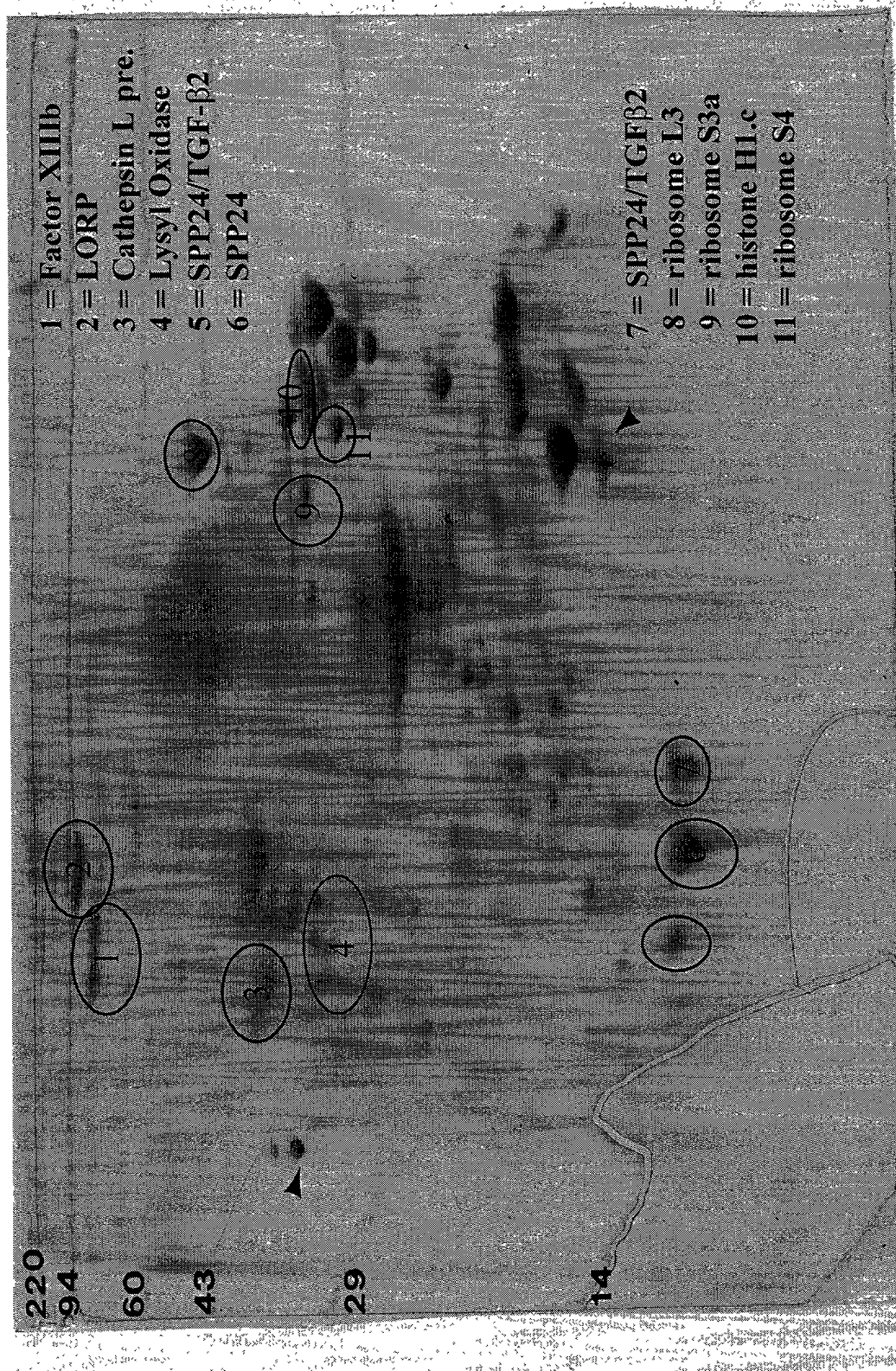


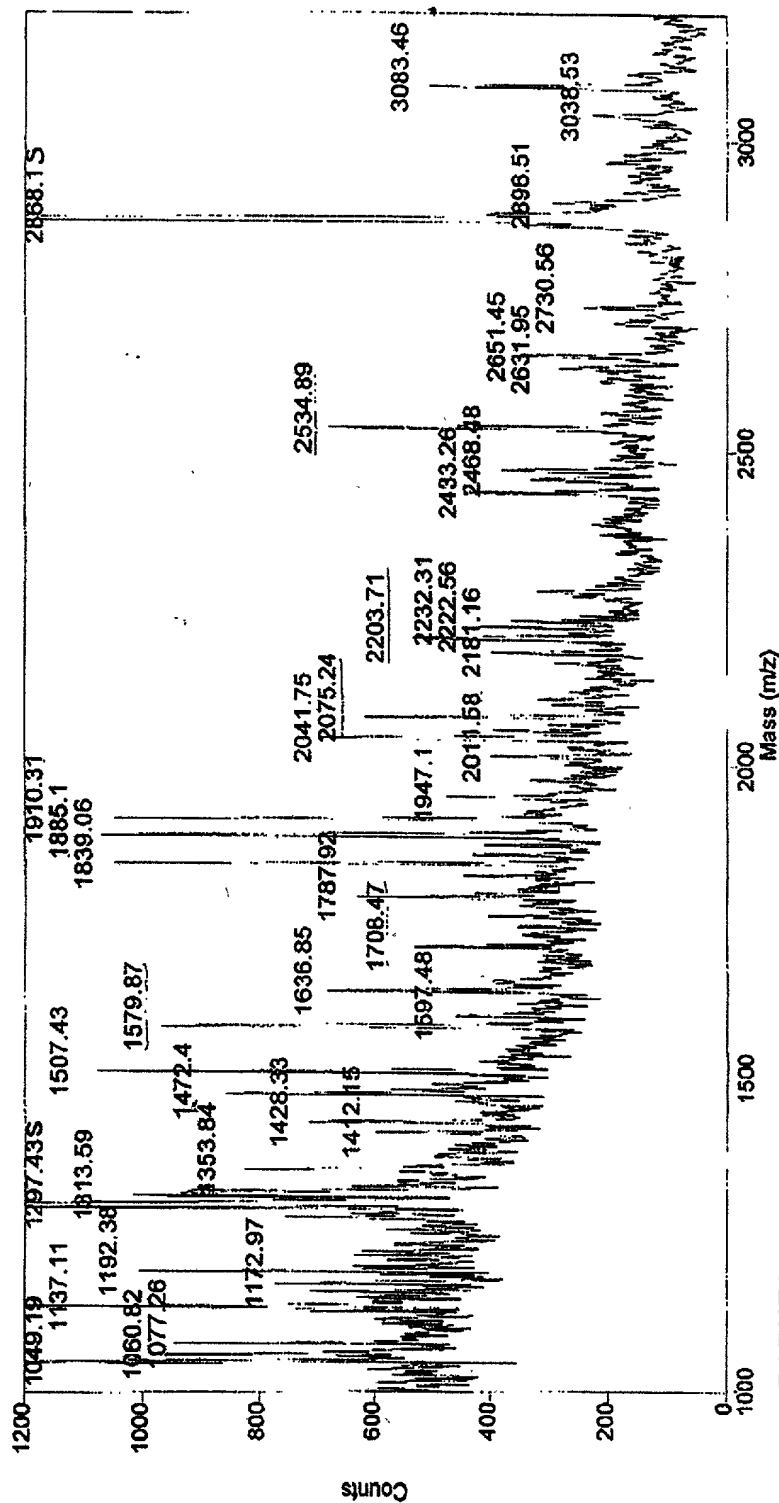
FIGURE 6

Figure 7A (Band 1)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1099\digest\barnes026.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/12/99 2:13 PM Sample: 74



Comment: BARNES-1, trypsin, 7.5 %

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 20000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Laser: 1965

Scans Averaged: 256

Pressure: 1.70e-06

Low Mass Gate: 500.0

Mirror Ratio: 1.060

PSD Mirror Ratio:

Timed Ion Selector: 16.1 OFF

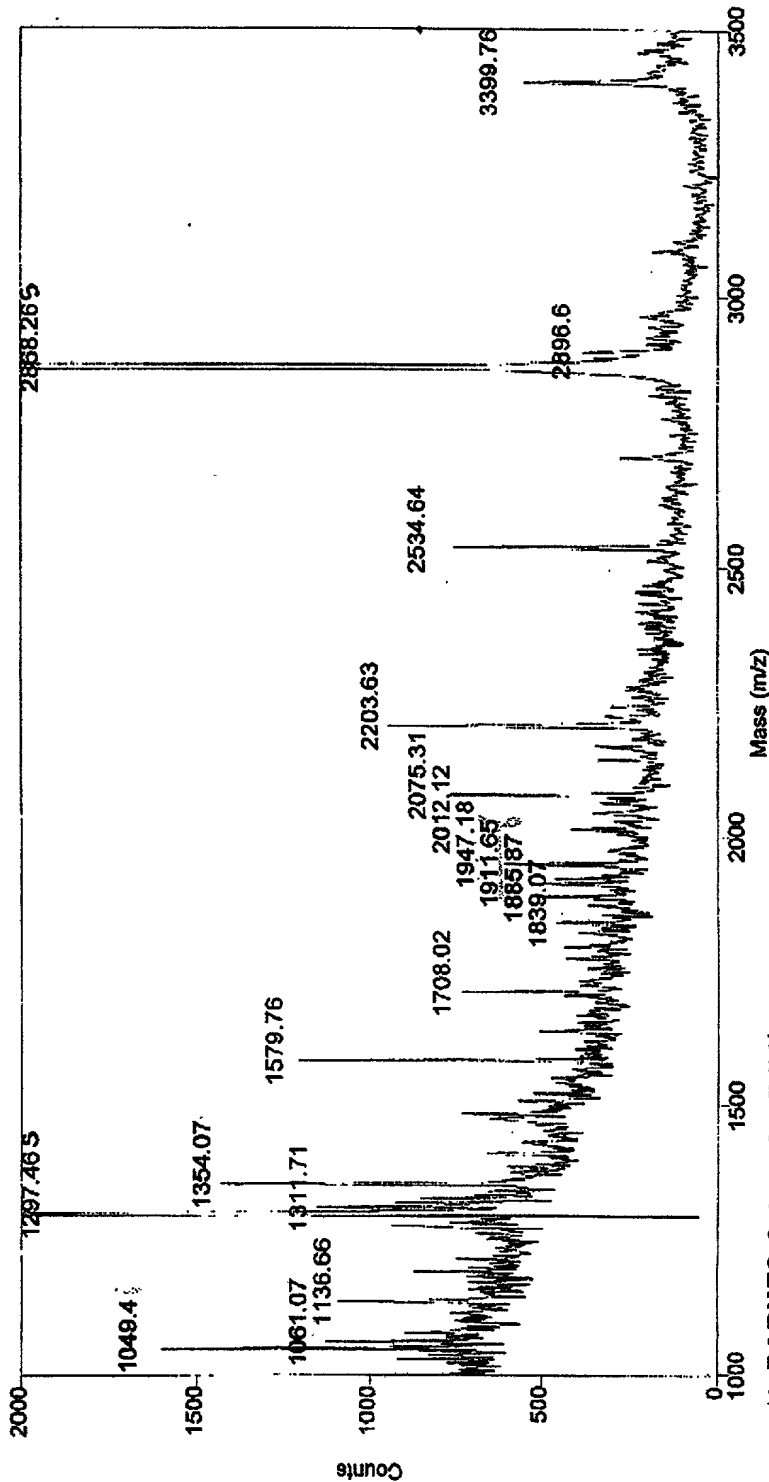
Negative Ions: OFF



Columbia University /HHMI Protein Core

Figure 7B (Band 2)

Original Filename: c:\voyager\data\mag1099\digest\barnes027.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/12/99 2:21 PM Sample: 75



Comment: BARNES-2, trypsin, 7.5 %

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 20000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Laser : 1965

Scans Averaged: 256

Pressure: 1.58e-06

Low Mass Gate: 500.0

Mirror Ratio: 1.060

PSD Mirror Ratio:

Timed Ion Selector: 16.1 OFF

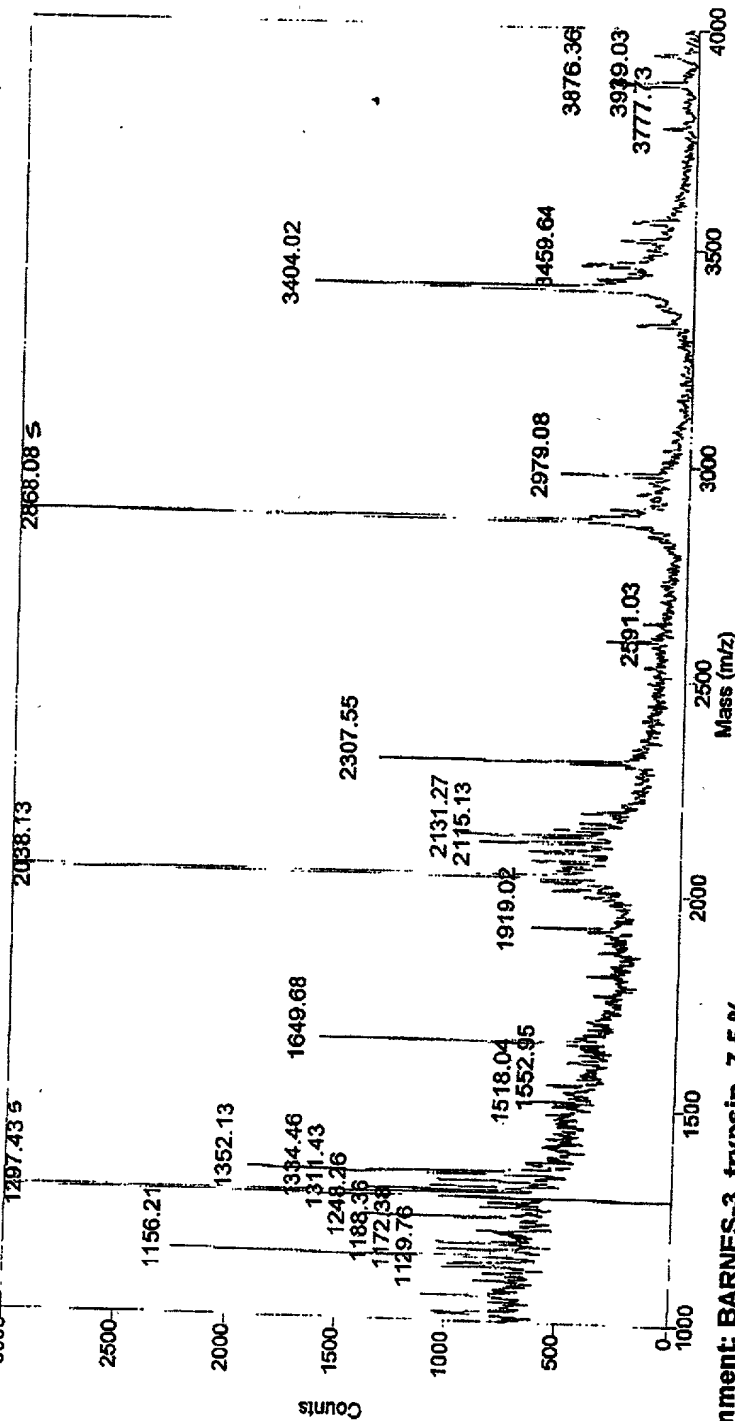
Negative Ions: OFF

Figure 7C (Band 3)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1099\digest\barnes028.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\digest\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/12/99 2:31 PM Sample: 76



Comment: BARNES-3, trypsin, 7.5 %

Method: LDE1000A
 Mode: Linear

Accelerating Voltage: 20000
 Grid Voltage: 94.000 %
 Guide Wire Voltage: 0.075 %
 Delay: 50 ON

Laser : 1965
 Scans Averaged: 256
 Pressure: 1.5⁻⁶ Torr
 Low Mass Gate: 500.0

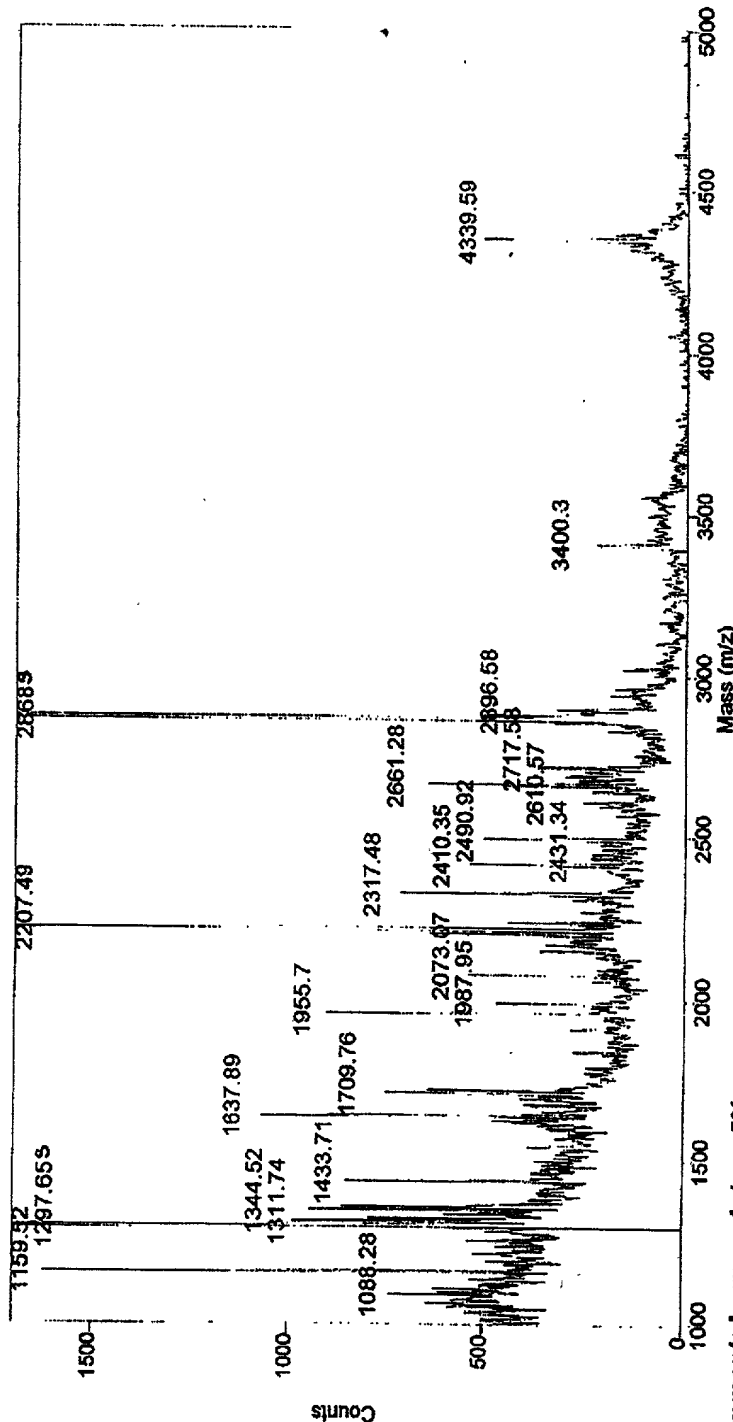
Mirror Ratio: 1.060
 PSD Mirror Ratio:
 Timed Ion Selector: 16.1 OFF
 Negative Ions: OFF

Figure 7D (Band 4)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1099\barnes\barnes037.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\BARNES\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/27/99 2:30 PM Sample: 22



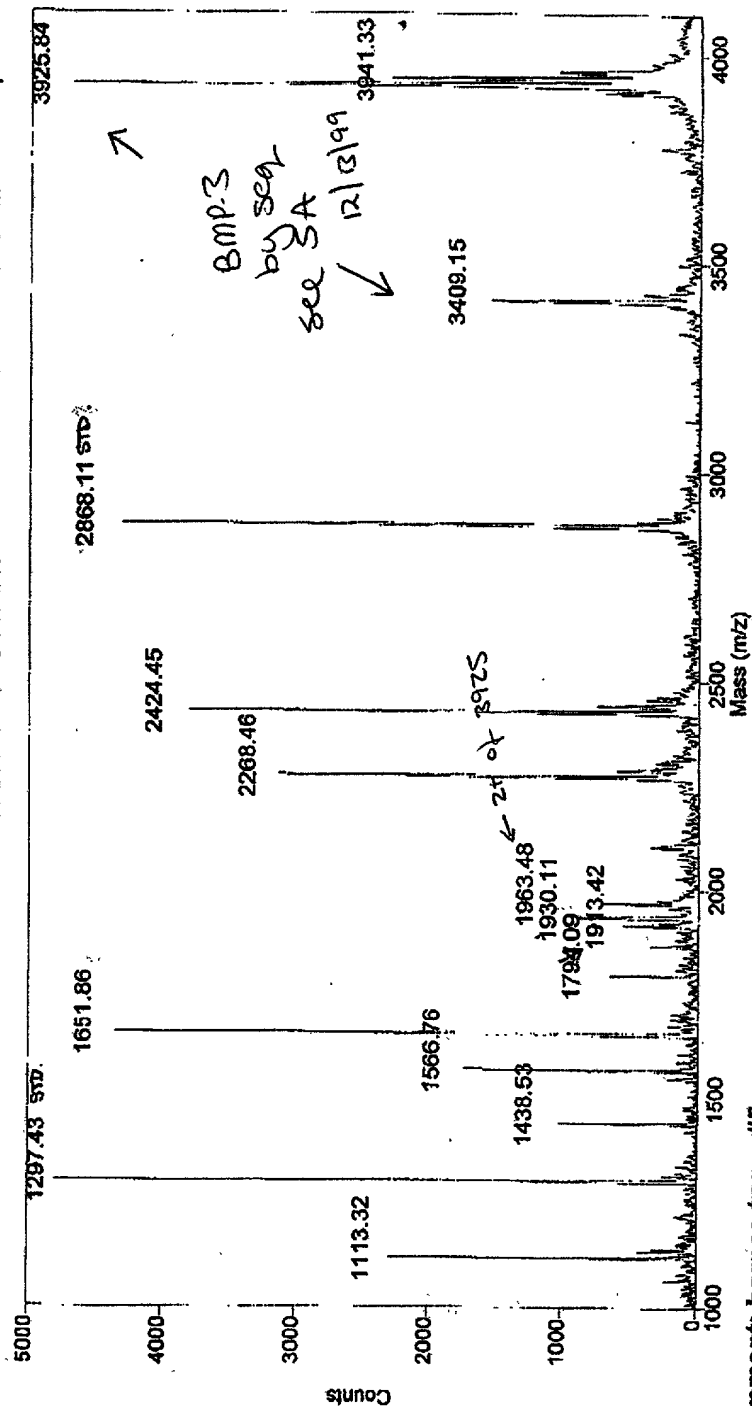
Comment: barnes-4, trys 5% S-Std.
 Method: LDE1000A Accelerating Voltage: 20000
 Mode: Linear Grid Voltage: 94.000 %
 Guide Wire Voltage: 0.075 %
 Delay: 50 ON
 Laser: 1965
 Scans Averaged: 256
 Pressure: 9.04e-07
 Low Mass Gate: 500.0
 Mirror Ratio: 1.060
 PSD Mirror Ratio:
 Timed Ion Selector: 16.1 OFF
 Negative Ions: OFF

Figure 7E (Band 5)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1099\digest\barname002.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/5/99 1:16 PM Sample: 32



Comment: barnes tryps #5

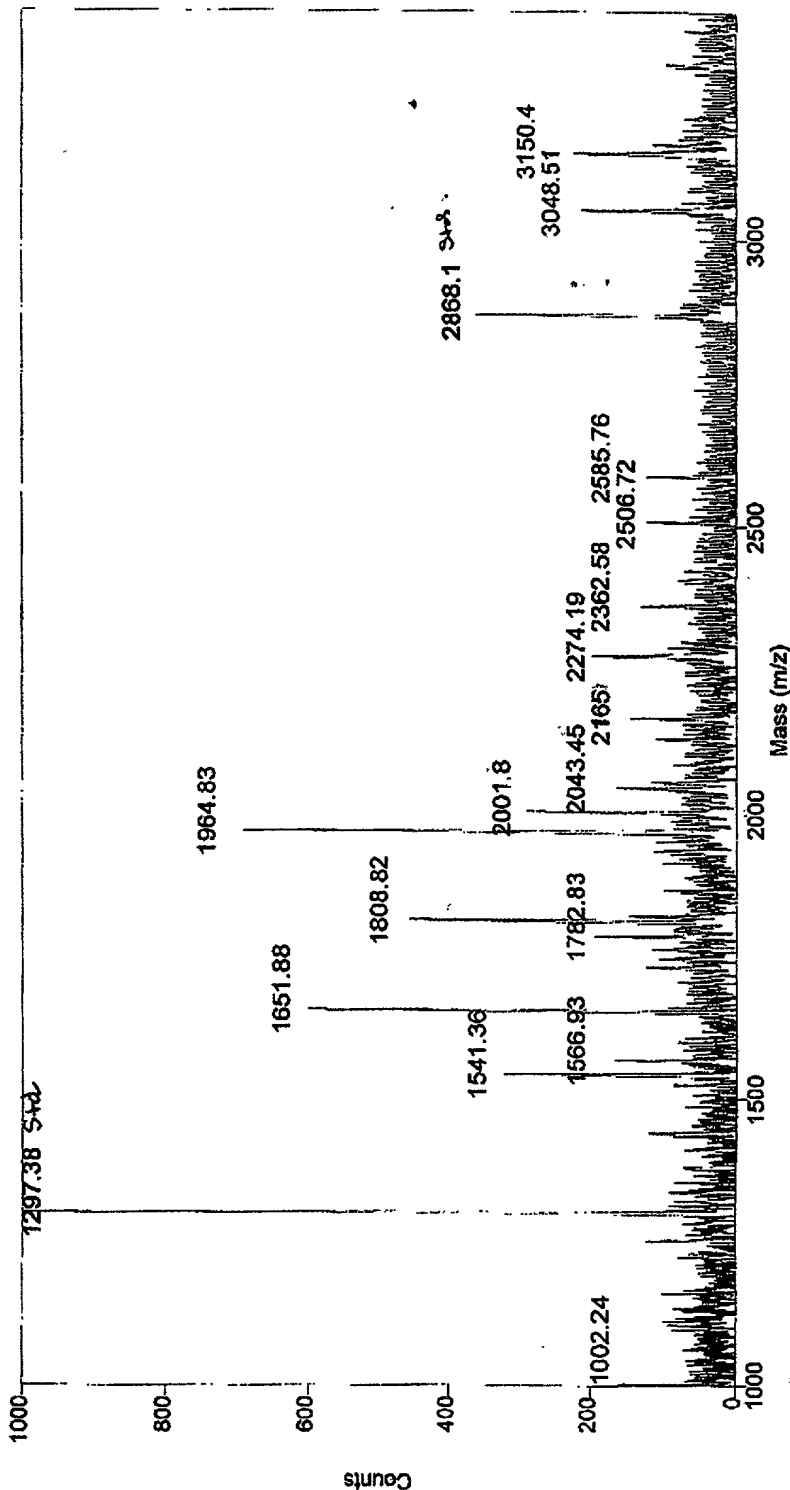
Method: LDE1000A	Accelerating Voltage: 20000	Laser: 1965	Mirror Ratio: 1.060
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 121	PSD Mirror Ratio:
	Guide Wire Voltage: 0.075 %	Pressure: 3.68e-07	Timed Ion Selector: 16.1 OFF
	Delay: 50 ON	Low Mass Gate: 500.0	Negative Ions: OFF



Columbia University /HHMI Protein Core

Figure 7F (Band 6)

Original Filename: c:\voyager\data\mag1099\digest\barnes001.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 10/5/99 1:14 PM Sample: 33



Comment: barnes tryps #6

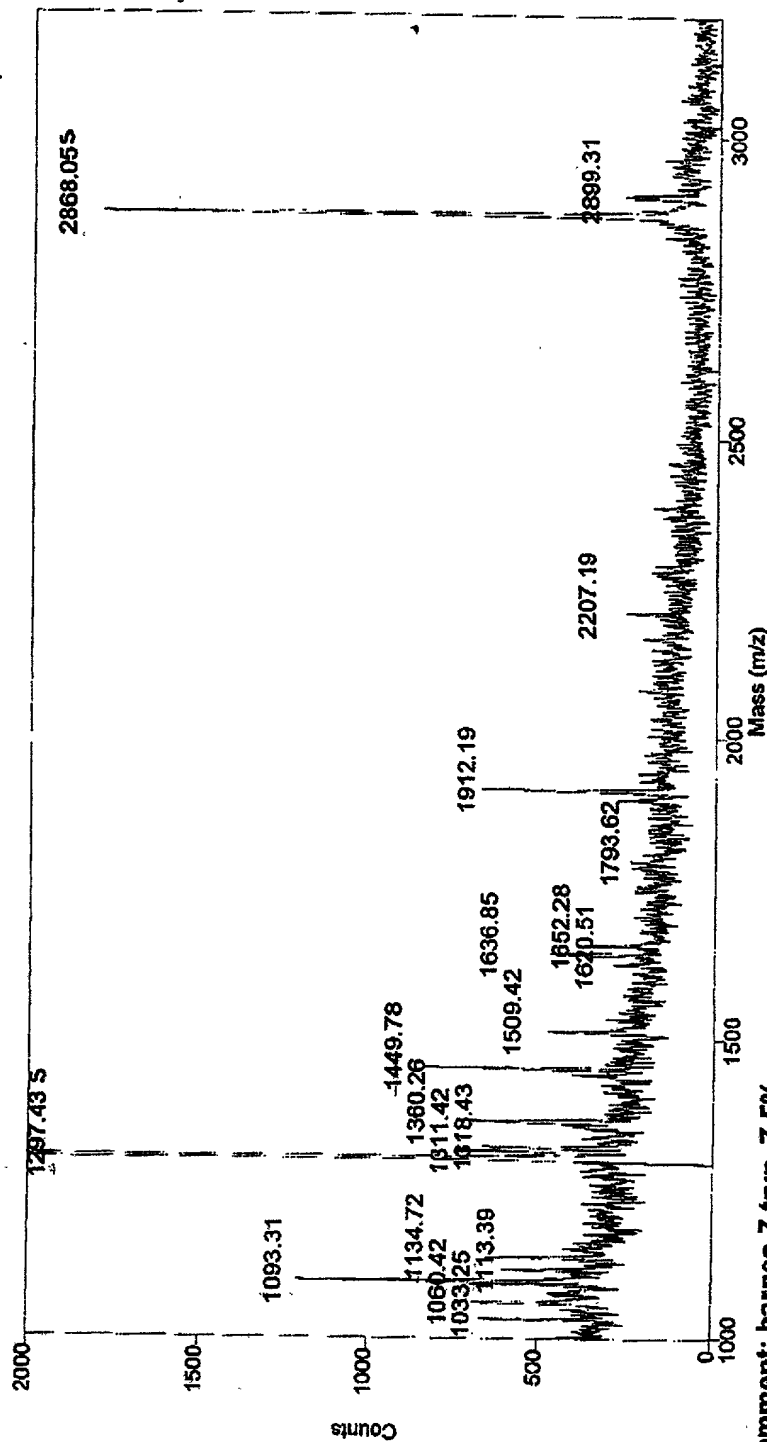
Method: LDE1000A	Accelerating Voltage: 20000	Laser: 1965	Mirror Ratio: 1.060
Mode: Linear	Grid Voltage: 94,000 %	Scans Averaged: 256	PSD Mirror Ratio:
	Guide Wire Voltage: 0.075 %	Pressure: 4.06e-07	Timed Ion Selector: 16.1 OFF
	Defav: 50 ON	Ion Mass Gate: 500 n	Max Ion Time: 0.07

Figure 7G (Band 7)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1199\digests\nrow_004.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1199\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 11/10/99 3:11 PM Sample: 65



Comment: barnes-7, tryp, 7.5%

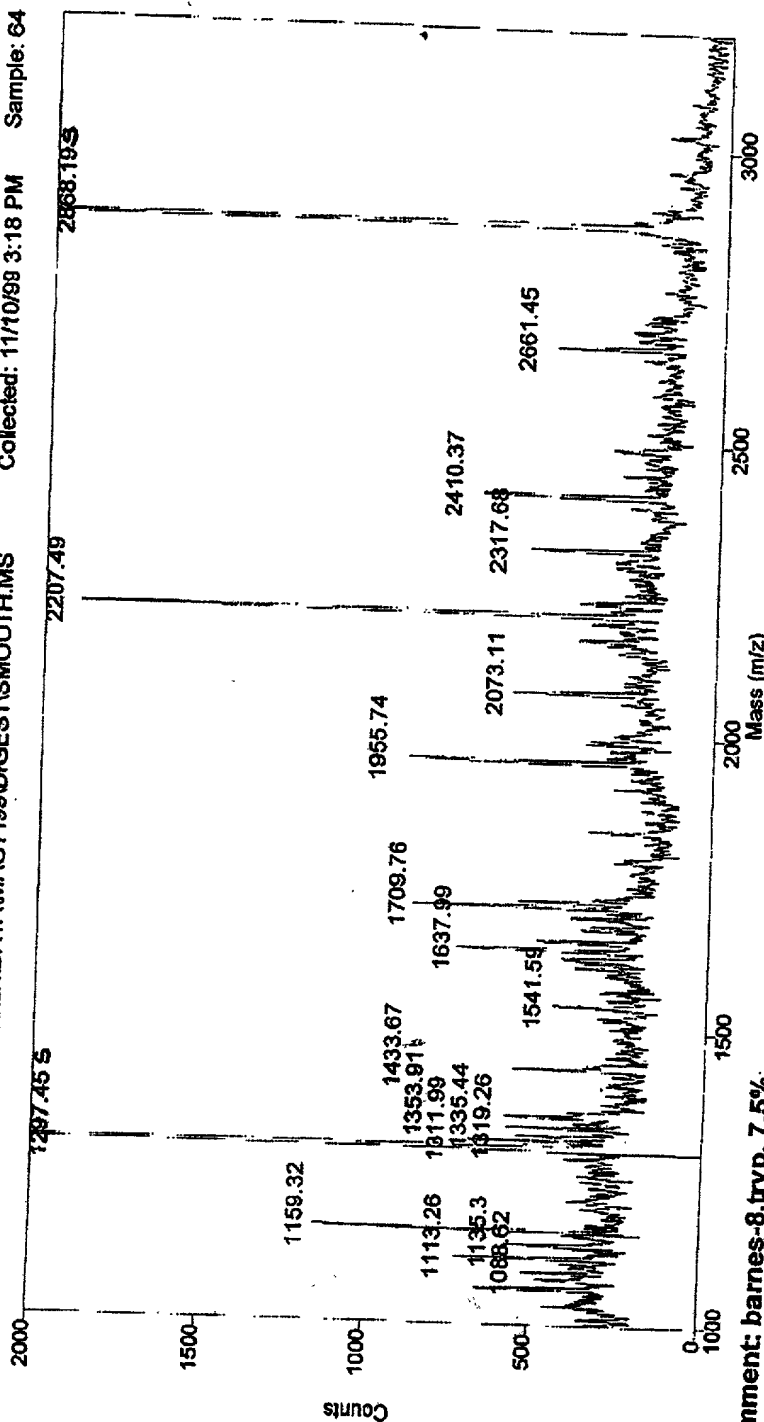
Method: LDE1000A	Accelerating Voltage: 20000	Laser: 1965	Mirror Ratio: 1.060
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 256	PSD Mirror Ratio:
	Guide Wire Voltage: 0.075 %	Pressure: 5.89e-07	Timed Ion Selector: 16" OFF
	Delay: 50 ON	Low Mass Gate: 500.0	Negative Ions: ON

Figure 7H (Band 8)



Columbia University /HHM Protein Core

Original Filename: c:\voyager\data\mag1199\digest\snw_005.ms
 This File # 1 : C:\VOYAGER\DATA\MAG1199\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 11/10/98 3:18 PM Sample: 64



Comment: barnes-8, try, 7.5%

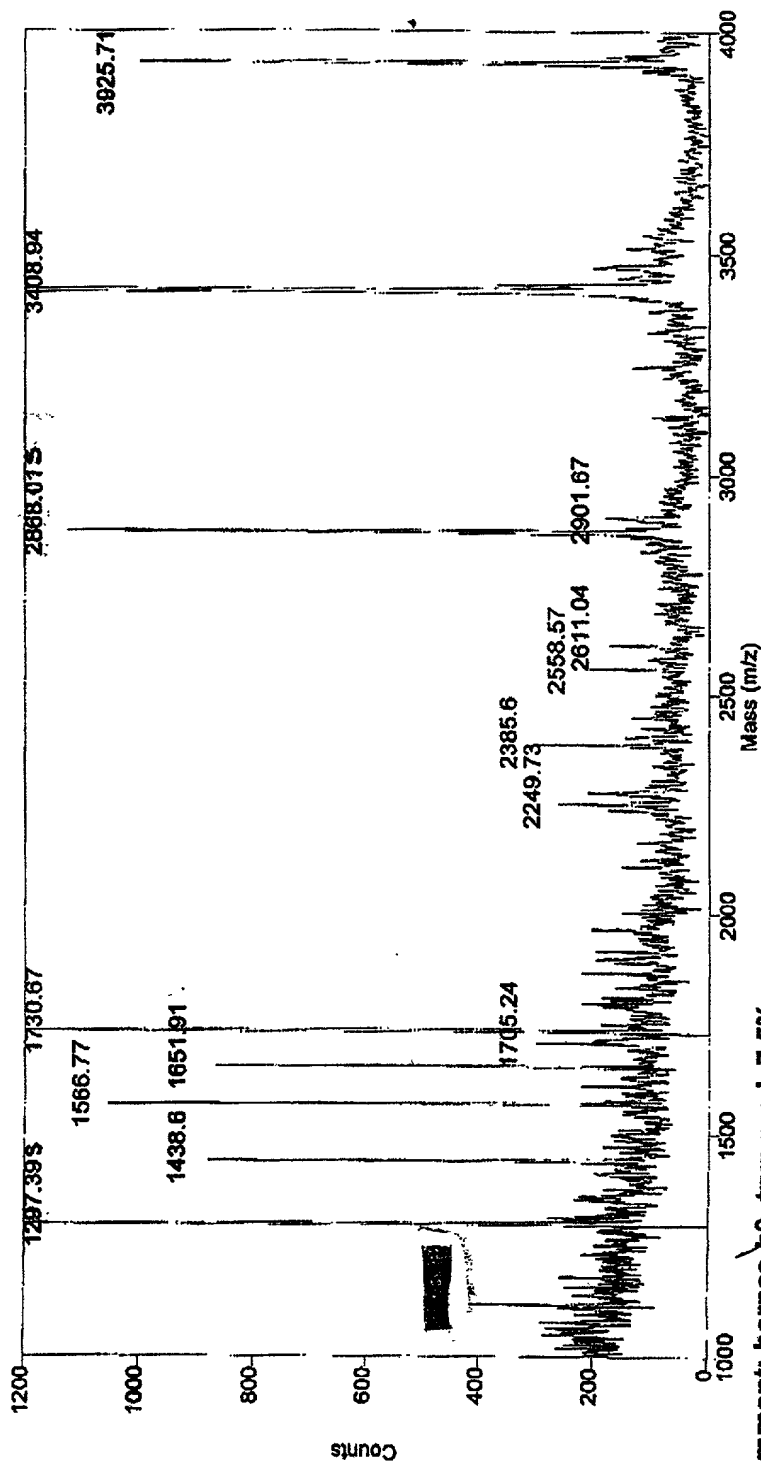
Method: LDE1000A
 Mode: Linear

Accelerating Voltage: 20000
 Grid Voltage: 94.000 %
 Guide Wire Voltage: 0.075 %
 Delay: 50 ON

Laser: 1965
 Scans Averaged: 256
 Pressure: 4.01e-07
 Low Mass Gate: 500.0

Mirror Ratio: 1.060
 PSD Mirror Ratio:
 Timed Ion Selector: 16.1 OFF
 Negative Ions: OFF

Savitsky-Golay Order = 2 Points = 19
Collected: 2/23/80 3:25 PM Sample: 44



Comment: barnes-b9, tryp.mod., 7.5%

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 25000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser: 1860

Scans Averaged: 256

Pressure: 3.27e-07

Low Mass Gate: 500.0

Mirror Ratio: 1.080

PSD Mirror Ratio:

Timed Ion Selector: 16.1 OFF

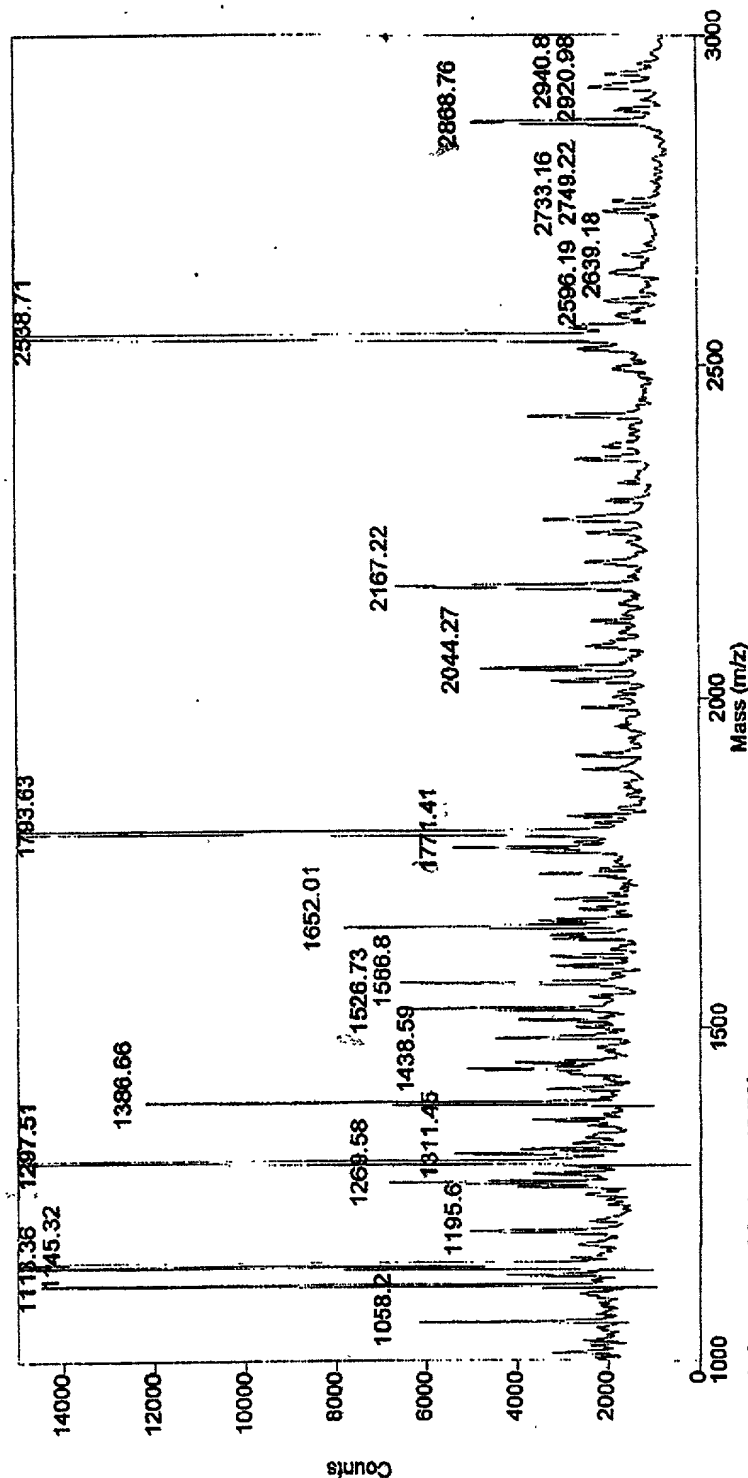
Negative Ions: OFF

Figure 7J (Band 11)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag1299\digest\barnes004.ms
 This File # 2 : C:\VOYAGER\DATA\MAG1299\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 12/15/99 3:49 PM Sample: 14



Comment: barnes-11, trypt, 7.5%

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 25000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser: 1965

Scans Averaged: 256

Pressure: 5.84e-07

Low Mass Gate: 500.0

Mirror Ratio: 1.080

PSD Mirror Ratio:

Timed Ion Selector: 1f 1 OFF

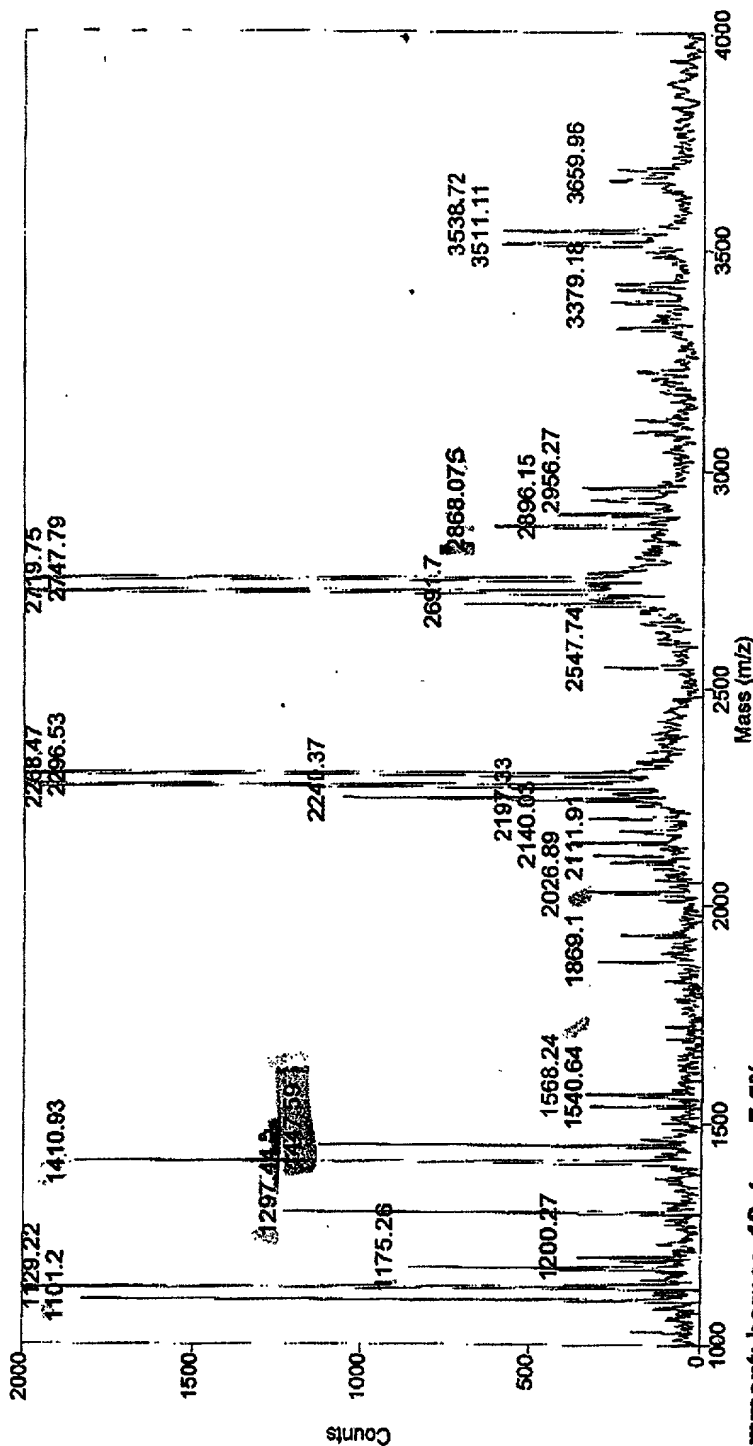
Negative Ions: 0.1

Figure 7K (Band 18)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\nag1299\digest\barnes005.ms
 This File # 2 : C:\VOYAGER\DATA\MAG1299\digest\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 12/15/99 4:47 PM Sample: 13



Comment: barnes-18, trypt, 7.5%

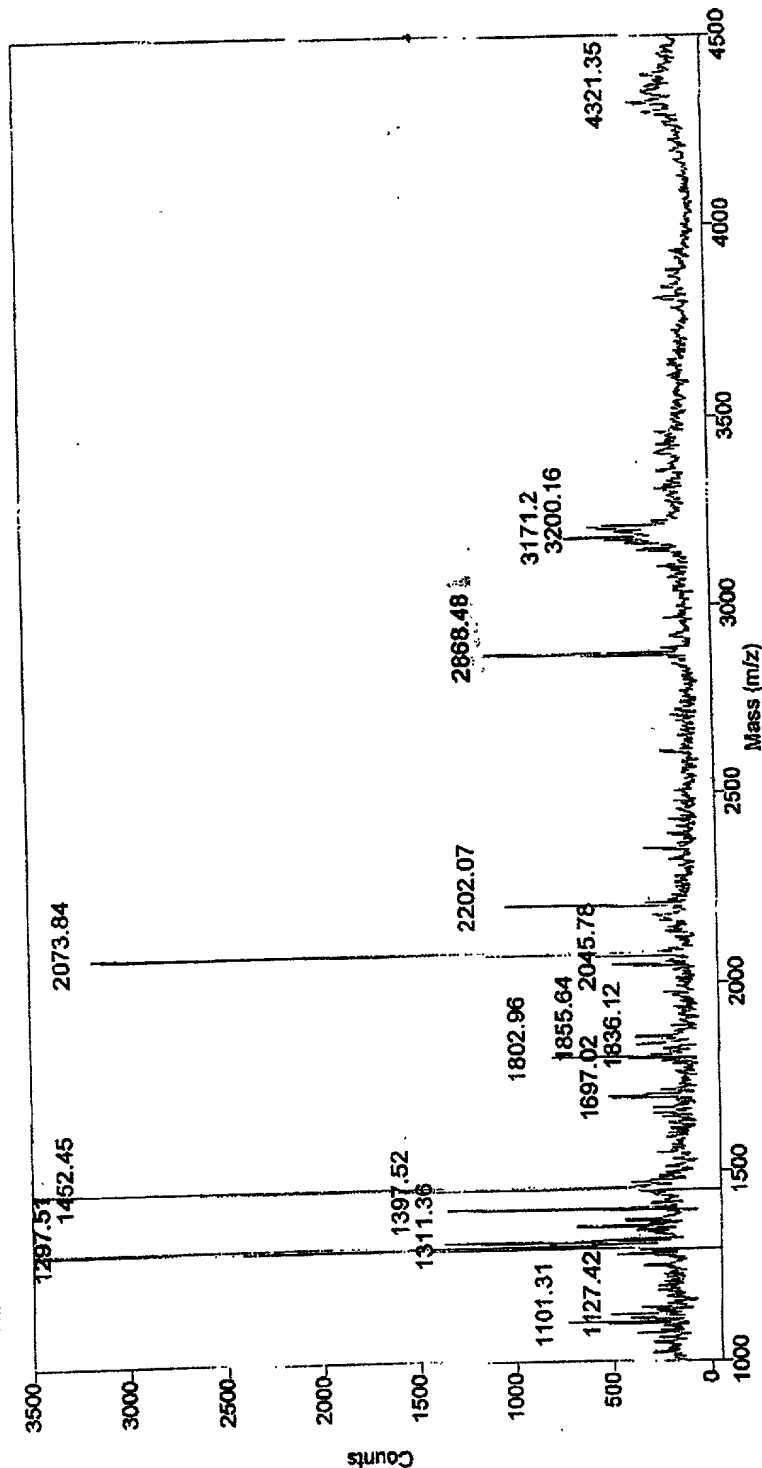
Method: LDE1000A	Accelerating Voltage: 25000	Laser: 1745	Mirror Ratio: 1.080
Mode: Linear	Grid Voltage: 94.000 %	Scans Averaged: 256	PSD Mirror Ratio:
	Guide Wire Voltage: 0.090 %	Pressure: 2.90e-07	Timed Ion Selector: 16.1 OFF
	Delay: 50 ON	Low Mass Gate: 500.0	Negative Ions: OFF

Figure 7L (Band 20)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag100\digest\lame001.ms
 This File # 4 : C:\VOYAGER\DATA\MAG100\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 1/6/80 3:36 PM Sample: 42



Comment: barnes-20, tryp, 7.5%

Method: LDE1000A
 Mode: Linear

Accelerating Voltage: 25000
 Grid Voltage: 94.000 %
 Guide Wire Voltage: 0.090 %
 Delay: 50 ON

Laser: 1820
 Scans Averaged: 256
 Pressure: 9.21e-07
 Low Mass Gate: 500.0

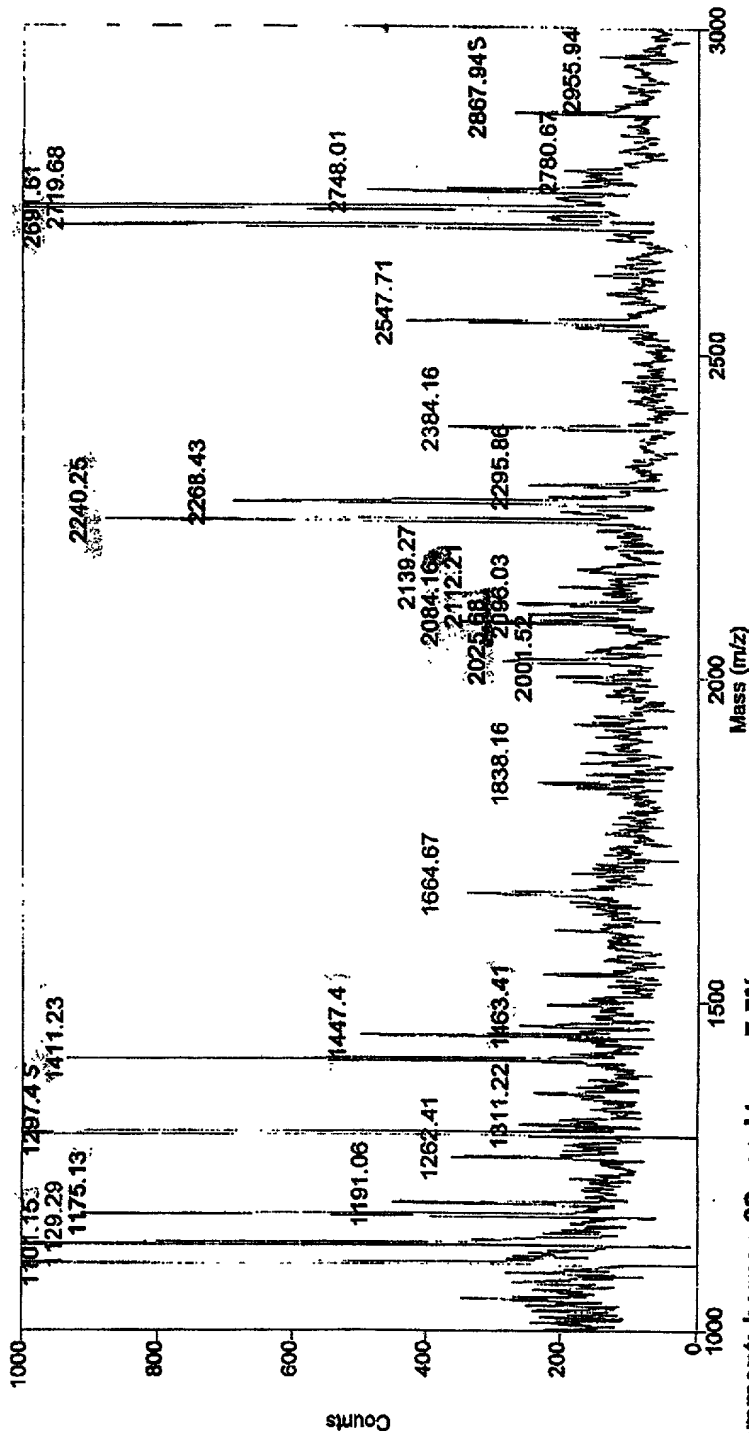
Mirror Ratio: 1.080
 PSD Mirror Ratio:
 Timed Ion Selector: 1p - OFF
 Negative Ions: OFF

Figure 7M (Band 22)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag200\digest\bame003.ms
 This File # 1 : C:\VOYAGER\DATA\MAG200\DIGEST\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 2/16/80 3:35 PM Sample: 54



Comment: barnes-22, mod.tryp., 7.5%

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 25000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser: 1870

Scans Averaged: 231

Pressure: 3.43e-07

Low Mass Gate: 500.0

Mirror Ratio: 1.080

PSD Mirror Ratio:

Timed Ion Selector: 16 ° OFF

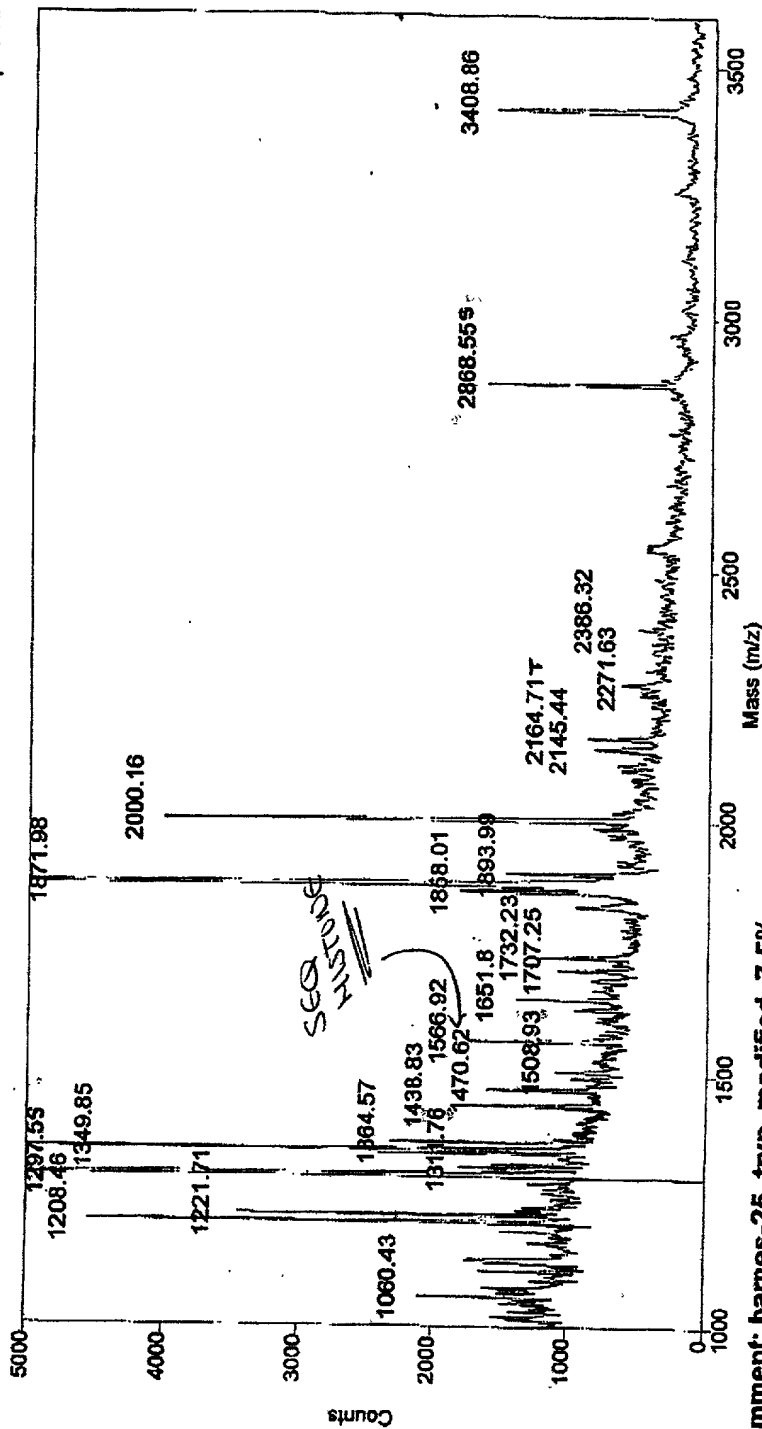
Negative Ions: ON

Figure 7N (Band 25)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag200\digest\barnes001.ms
 This File # 2 : C:\VOYAGER\DATA\MAG200\digest\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 2/2/80 3:24 PM Sample: 62



Comment: barnes-25, tryp. modified, 7.5%

Method: LDE1000A
 Mode: Linear

Accelerating Voltage: 25000
 Grid Voltage: 94.000 %
 Guide Wire Voltage: 0.090 %
 Delay: 50 ON

Laser: 1880
 Scans Averaged: 256
 Pressure: 9.26e-07
 Low Mass Gate: 500.0

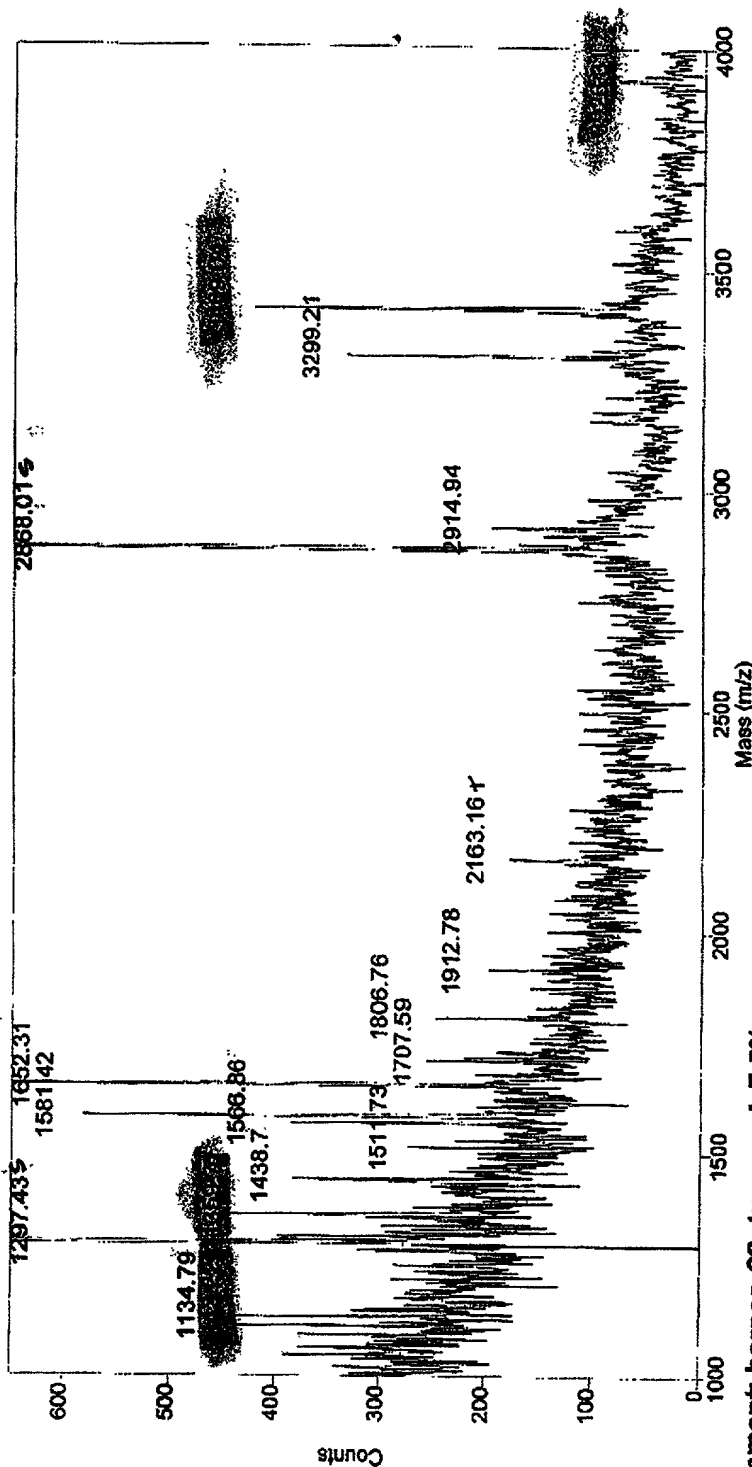
Mirror Ratio: 1.080
 PSD Mirror Ratio:
 Timed Ion Selector: 1p OFF
 Negative Ions: Off

Figure 70 (Band 29)



Columbia University /HHMI Protein Core

Original Filename: c:\voyager\data\mag200\digest\barnes006.ms
 This File # 2 : C:\VOYAGER\DATA\MAG200\digest\SMOOTH.MS
 Savitsky-Golay Order = 2 Points = 19
 Collected: 2/23/80 3:19 PM Sample: 43



Comment: barnes-29, tryp.mod., 7.5%

Method: LDE1000A

Mode: Linear

Accelerating Voltage: 25000

Grid Voltage: 94.000 %

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser : 1860

Scans Averaged: 256

Pressure: 3.82e-07

Low Mass Gate: 500.0

Mirror Ratio: 1.080

PSD Mirror Ratio:

Timed Ion Selector: 16.1 OFF

Negative Ions: OFF

097483 0400 3E08460

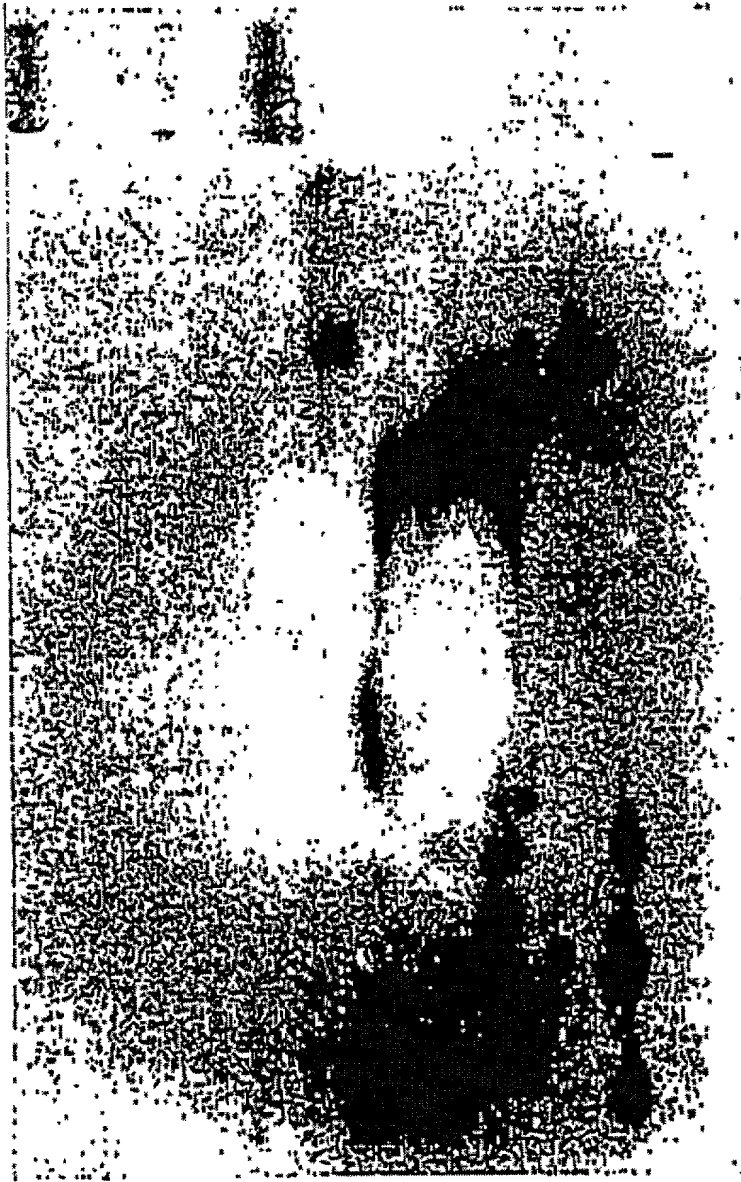


Figure 8

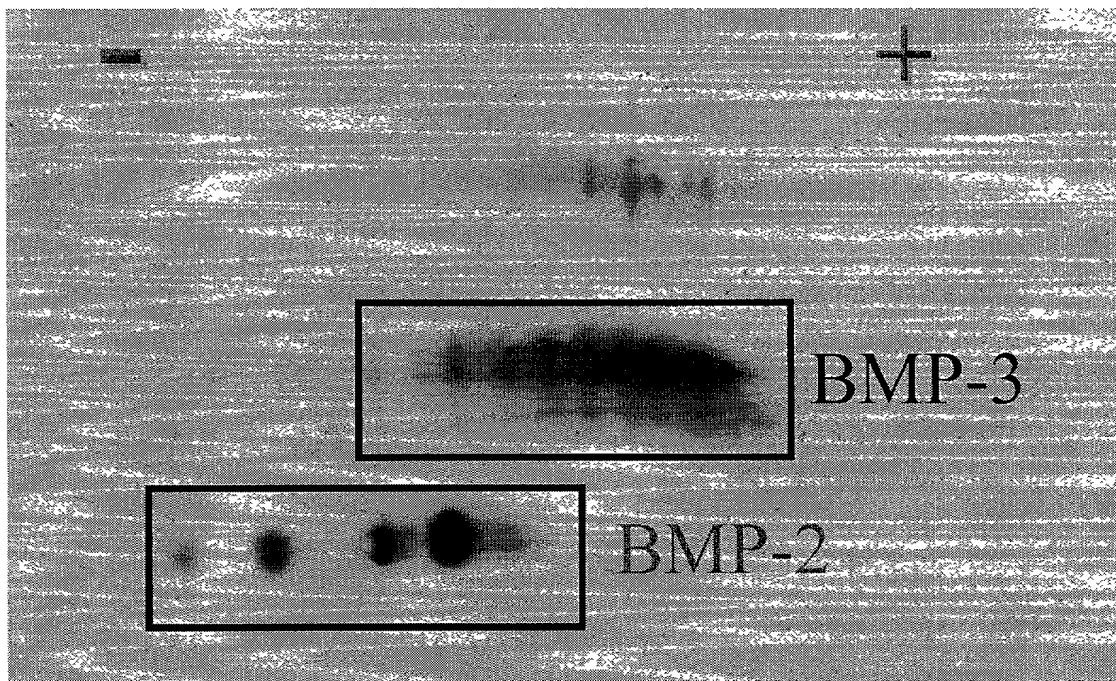


FIGURE 9A

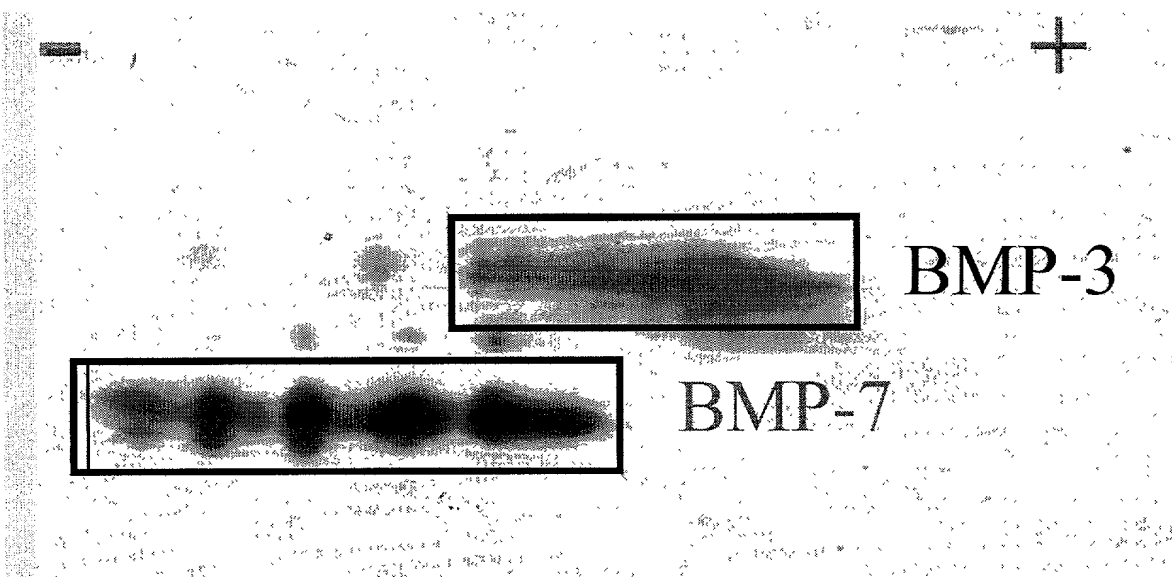


FIGURE 9B

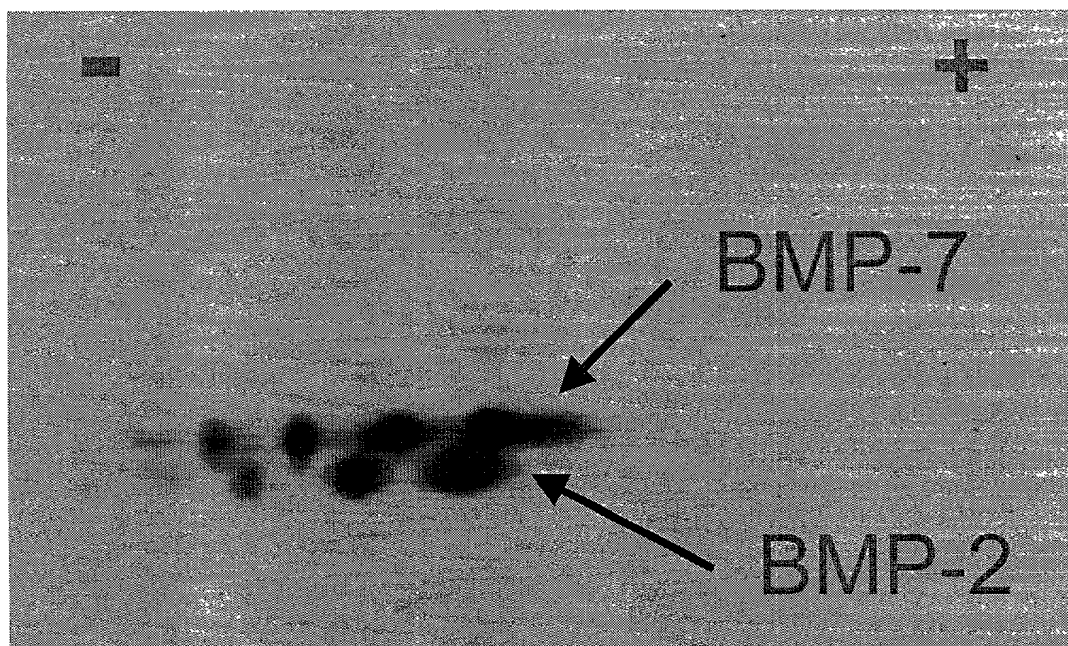


FIGURE 9C

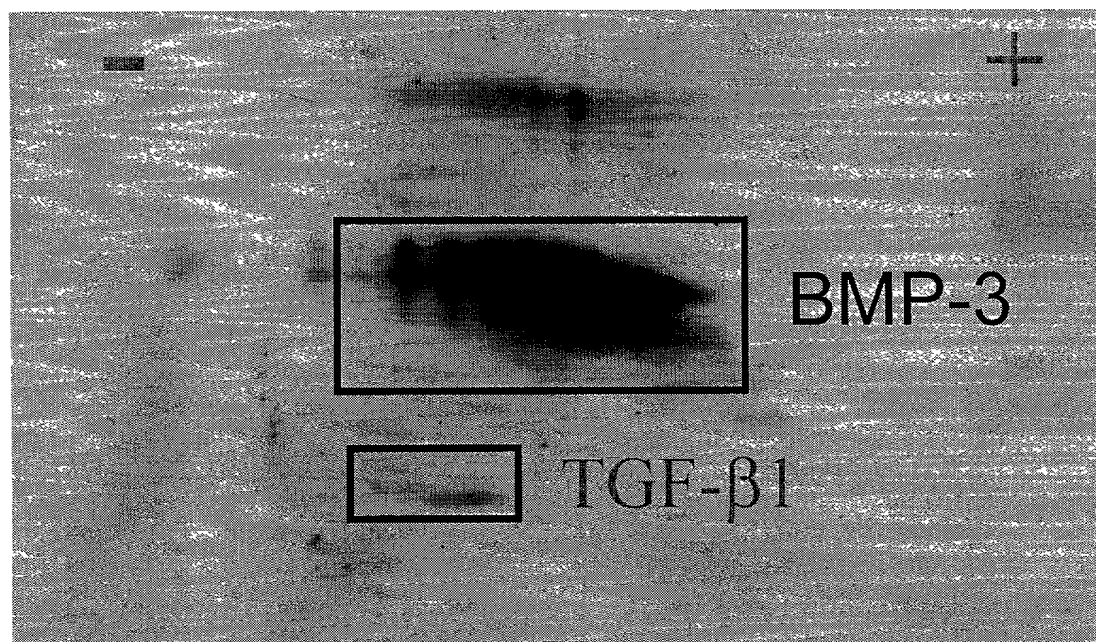
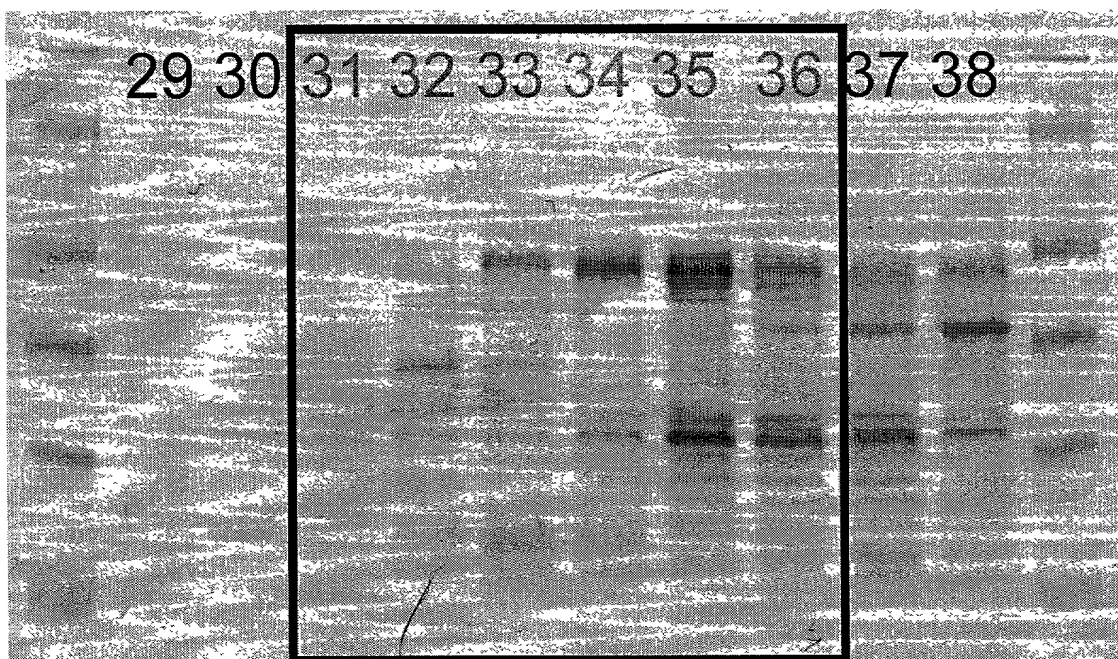


FIGURE 9D

FIGURE 10



- + + + + - + + + +



FIGURE 11

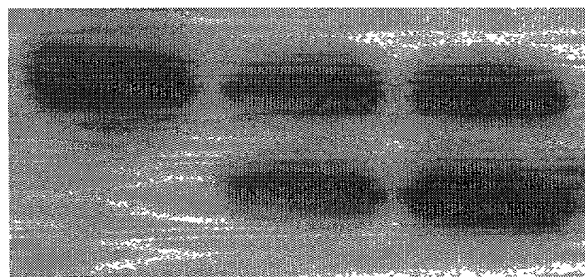


FIGURE 12

FIGURE 13A

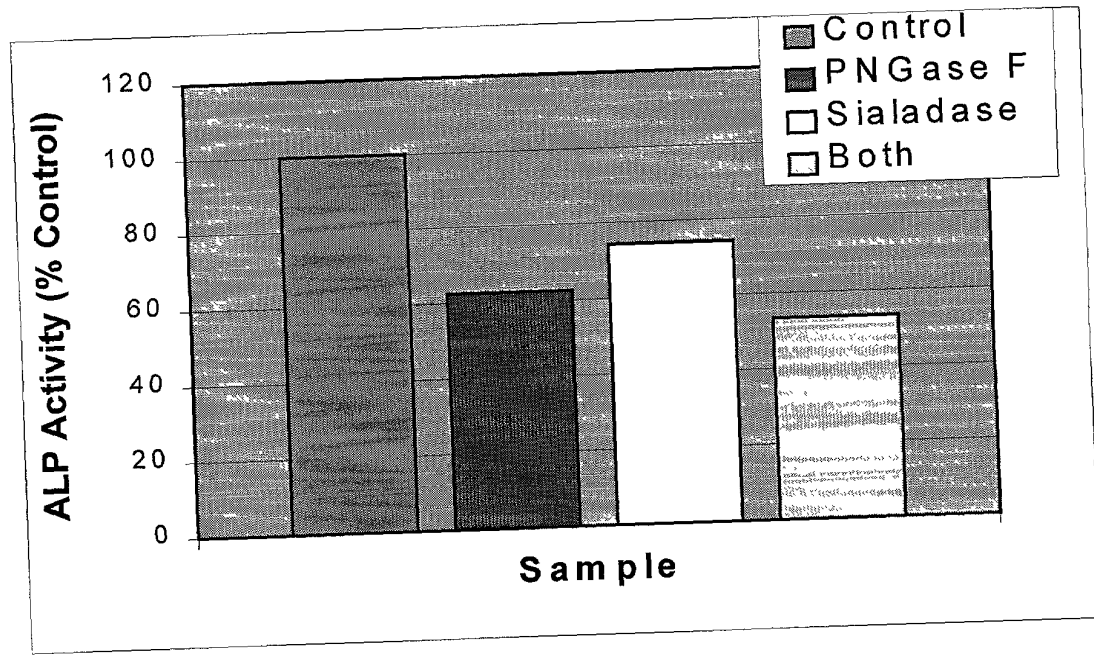
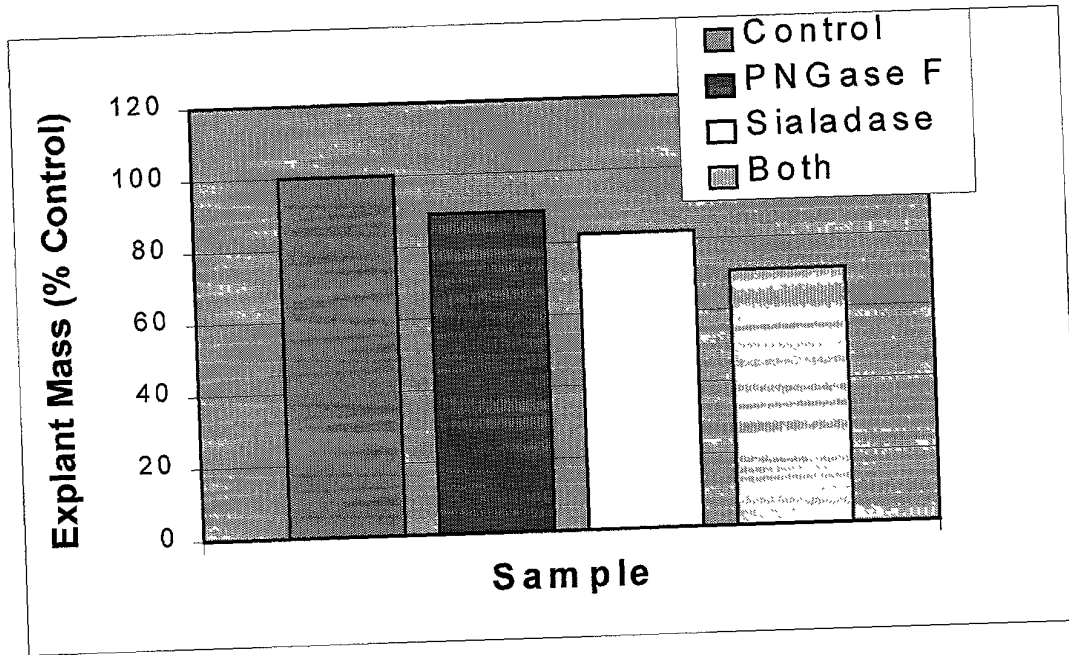


FIGURE 13B

FIGURE 14: Antibody Listing

Specificity	Antigen	Host Species	PC/MC	Source	Catalog No.
TGF- β 1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221
TGF- β 2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90
TGF- β 3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	so-6896
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novocastra Laboratories	NCL-BMP6
BMP-7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA
FGF-1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884
osteonectin (bovine)	Protein	Mouse	Monoclonal	DSHB	AON-1
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R1H
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740

Figure 15A: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
1								
2	fx 49 (1579)	XLAAGYDVEK	ALAAAGYDVEK	11/11	histone H1.c	human	87668 (NCBI)	65-75
3	fx 67 (1346)	SLEKVCADLR	SLEKVCADLR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4	fx 65 ()	(V)VCGMLGFPSEAPV	VCGMLGFPGEKRV	11/14	LORP	mouse	AAC96338 (NCBI)	213-226
5	N terminal seq	STGVLLPLQNNELPG	STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290-304
	fx 72 (3925)	STGVLLPLQNNELPGAQYQY	STGVLLPLQNNELPGAQYQY	20/20	BMP-3	human	4557371 (NCBI)	290-309
	fx 74 (3409)	STGVLLPLQ	STGVLLPLQ	9/9	BMP-3	human	4557371 (NCBI)	290-298
6	fx 55 (1566)	(S)QTLQFXE	SQTLQFDE	7/8	BMP-3	human	4557371 (NCBI)	346-353
	fx 47	VYAF	no match		???			
	N terminal seq	HAGKYSREKNT(P)A(P)	HGGKYSREKNQPKP	11/14	α 2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46
	fx 57 (1438)	SQTLQFDEQ	SQTLQFDEQ	9/9	BMP-3	human	4557371 (NCBI)	346-354
	fx 57 (1652)	SLKPSNHA	SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410-417
7	fx 51 (1093)	AALRPLVKP	AALRPLVKP	9/9	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	1-9
	fx 37 (no MS)	A(H)(Q)VERYV	AIVER	5/5	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	109-113
	fx 37 (no MS)	A(H)(Q)VERYV	HQSDRYV	5/7	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	22-28
8	fx 78 ()	XALF(G)AQLGXALGPI	no match		???			
9	fx 56 (1567)	SQTLQFDEQT	SQTLQFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346-355

Figure 15B: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
11	fx 55 (1311)	SQTLXF	SQTLQF	5/6	BMP-3	human	4557371 (NCBI)	346-351
	fx 47 (1772)	VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	87-99
	fx 76 (1795)	xVFAL	VFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
	fx 61 (1145)	AVPQLQGYLR	AIPQLQGYLR	9/10	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	262-271
18								
22	fx 58 (1101)	ALDAAYCFR	ALDAAYCFR	9/9	TGF-β2	human	P08112 (Swiss-Prot)	303-311
	fx 69 (no match)	GYNANFCAGACPYL	GYNANFCAGACPYL	14/14	TGF-β2	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71)	VNSQSLSPY	VNSQSLSPY	9/9	SPP24	bovine	Q27967 (Swiss-Prot)	42-50
25	fx 39 (1470)	KAAPSPV(P)	KAAPSPV	8/8	Histone H1 x	human	JC4928 (PIR)	199-206
29								

fx=fraction number (molecular weight of fragment, as measured by SDS-PAGE)

Figure 16A: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
1	4 peaks match with histone H1.c	human	87668 (NCBI)	1172.97	1172.37	0.60	110-121	22	15 MS peaks match with Band 2
				1579.87	1579.71	0.16	65-79		
				1708.47	1707.89	0.58	64-79		
				2011.58	2012.32	-0.74	35-54		
2	3 peaks match with histone H1.c	human	87668 (NCBI)	1579.76	1579.71	0.05	65-79*	16	identification of starred peptide confirmed by sequence analysis
				1708.02	1707.89	0.13	64-79		
				2012.12	2012.32	-0.20	35-54		
				1129.76	1129.40	0.36	50-59		
3	7 peaks match with ribosome S20	rat	R3RT20 (PIR)	1156.21	1156.30	-0.09	76-83	62	
				1334.46	1334.62	-0.16	56-66		
				1352.13	1351.58	0.55	88-99		
				1518.04	1517.77	0.27	9-21		
				1919.02	1919.19	-0.17	5-21		
				3404.02	3404.87	-0.85	88-119		
				1987.95	1988.27	-0.32	150-167		
4	3 peaks match with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	2410.35	2410.63	-0.28	648-669	6	12 MS peaks match with Band 8
				2610.57	2610.10	0.47	455-478		

Figure 16B: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
5	9 peaks match with BMP-3	human	4557371 (NCBI)	1113.32	1113.31	0.01	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1438.53	1438.58	-0.05	346-357		
				1566.76	1566.76	0.00	345-357		
				1651.86	1651.91	-0.05	410-424		
				1794.09	1794.02	0.07	346-360		
				2268.46	2268.63	-0.17	374-392		
				2424.45	2424.81	-0.36	373-392		
6	3 peaks match with α 2-Macroglobulin RAP 2 peaks match with BMP-3	human	P30533 (Swiss-Prot)	3409.15	3407.77	1.38	290-318*	17	Identification of starred peptide confirmed by sequence analysis
				1002.24	1002.15	0.09	283-290		
				2362.58	2362.43	0.15	129-150		
				3048.51	3048.52	-0.01	257-282		
				1566.93	1566.75	0.18	346-357		
				1651.88	1651.91	-0.03	410-424		
			4557371 (NCBI)					15	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)

Figure 16C: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
7	4 peaks match with ribosome L32	mouse	P17932 (Swiss-Prot)	1033.25	1033.17	0.08	67-75	33	
				1093.31	1093.40	-0.09	1-10*		
				1134.72	1134.28	0.44	65-74		
				1449.78	1449.66	0.12	19-29		
				1060.42	1060.20	0.22	102-111		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.39	1113.31	0.08	361-368	21	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1360.26	1360.58	-0.32	190-200		
				1652.28	1651.91	0.37	410-424		
				1793.62	1794.02	-0.40	346-360		
				2410.37	2410.63	-0.26	648-669		
8	1 peak matches with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)					3	12 MS peaks match with Band 4
9	6 peaks match with BMP-3	human	4557371 (NCBI)	1113.14	1113.31	-0.17	361-368	36	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1438.60	1438.58	0.02	346-357		
				1566.77	1566.76	0.01	345-357		
				1651.91	1651.61	0.30	410-424		
				2901.67	2901.19	0.48	41-66		
				3408.94	3407.77	1.17	290-318		

Figure 16D: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
11	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.23	1113.31	-0.08	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1651.73	1651.91	-0.18	410-424		
				1793.58	1794.02	-0.44	346-360		
				2424.24	2424.81	-0.57	373-392		
				3408.34	3407.77	0.57	290-318		
18	5 peaks match with ribosome L6	human	Q02878 (Swiss-Prot)	1140.38	1140.23	0.15	114-122	16	
				1526.88	1526.86	0.02	141-155		
				1059.15	1059.12	0.03	10-20		
				1145.36	1145.35	0.01	262-271		
	4 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1386.74	1386.68	0.06	260-271	52	
				1101.20	1101.26	-0.06	303-311		
				1175.26	1175.42	-0.16	400-409		
				2240.37	2240.60	-0.23	312-328		
	5 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	2691.70	2691.91	-0.21	340-362	30	
				1410.93	1411.60	-0.67	42-53		
				1447.59	1447.65	-0.06	113-124		
				1540.64	1540.60	0.04	86-98		
				1869.10	1869.05	0.05	62-77		
				2268.47	2268.57	-0.10	33-53		

Figure 16E: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
22	5 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1101.15	1101.26	-0.11	303-311	63	
				1175.13	1175.42	-0.29	400-409		
				2084.16	2084.42	-0.26	312-347		
				2240.25	2240.60	-0.35	312-328		
				2691.61	2691.91	-0.30	340-362		
25	2 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1411.23	1411.60	-0.37	42-53	11	
				1447.40	1447.65	-0.25	113-124		
	5 peaks match with histone H1.x	human	JC4928 (PIR)	1208.46	1208.40	0.06	48-57	14	
				1221.71	1222.35	-0.64	107-118		
				1349.85	1350.52	-0.67	107-119		
				1364.57	1364.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
				1060.43	1060.20	0.23	102-111		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1438.83	1438.58	0.25	346-357	31	% coverage calculation is relative to the mature BMP-3, 183 AAs (280-472)
				1566.92	1566.76	0.16	345-357		
				1651.80	1651.91	-0.11	410-424		
				3408.86	3407.77	1.09	290-318		

% coverage calculation is relative to the mature BMP-3,183 AAS (290-472)

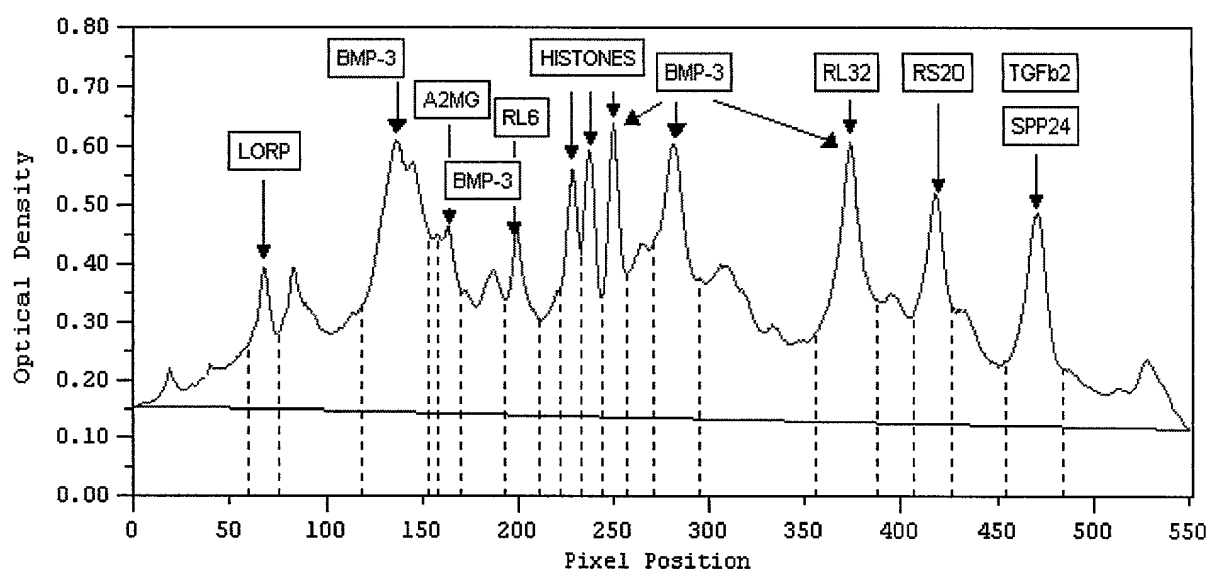


Figure 17A

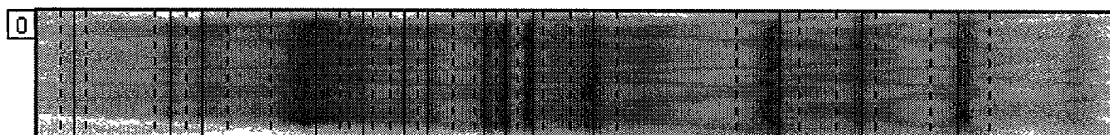


Figure 17B

FIGURE 18: Quantitation of Identified BP proteins

Identified Protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMP-3 and A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS2D	5
SPP24 & TGF- β 2	6
Total	58%

Figure 19A

Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			RAS	% Coverage	Comments
					Data	Database	Diff			
1	Lys-C	2 peaks match with Coagulation Factor XIIIb	Human	P05180 (Swiss-Prot)	1837.01	1837.14	-0.13	472-487	8	
					1921.85	1921.14	0.51	362-382		
					2679.51	N/A	N/A	438-504		peptide match confirmed by sequence analysis
2	Trypsin	2 peaks match with LORP	Human	NP002308 (Swiss-Prot)	1609.57	1609.88	-0.31	241-253	5	
					2410.88	2410.63	0.25	848-858		
3	Lys-C	8 peaks match with Cx36.1 precursor	Bovine	P25075 (Swiss-Prot)	1497.26	1495.80	1.46	105-116	41	
					1546.04	1548.70	-0.14	58-70		
					1881.18	1860.80	0.38	21-33		
					1881.46	1880.80	1.06	301-314		
					1834.71	1834.00	0.71	318-334		
					2352.80	2351.50	1.40	274-285		
					2381.50	2380.70	0.80	239-261		
					2721.51	2721.10	0.41	131-154		

Figure 19C
Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
7	Lys-C	4 peaks match with TGF- β 2	Bovine	P21214 (Swiss-Prot)	774.56	774.80	-0.34	26-31	42	
					809.69	808.94	-0.25	32-37		
					1175.92	1175.43	-0.31	88-107		
					3168.10	3166.68	1.44	1-25		
					2187.77	2187.51	0.26	42-80		
8	Trypsin	12 peaks match with ribosome L3	Bovine	P38872 (Swiss-Prot)	917.39	917.14	0.25	340-355	37	
					984.23	984.15	0.08	10-18		
					1192.62	1192.40	0.22	286-296		
					1380.67	1380.65	0.02	249-260		
					1484.80	1484.63	0.17	103-114		
					1620.06	1620.82	0.04	103-115		
					1778.84	1770.00	-0.16	94-99		
					2238.43	2238.55	-0.12	30-49		
					2325.99	2325.65	0.34	177-197		
					2681.31	2681.04	0.27	200-223		
					2697.94	2698.43	-0.49	70-90		
					2946.10	2948.35	-0.25	190-223		

Figure 19D
Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Defa	Database	Diff			
9	Trypsin	7 peaks match with ribosome S34	Mouse	P97351 (Swiss-Prot)	920.05	920.10	-0.05	19-25	29	
					1216.29	1216.31	-0.02	152-161		
					1346.62	1346.49	0.13	151-161		
					1516.69	1516.69	0.00	174-186		
					1593.72	1593.82	-0.10	94-108		
					1719.91	1720.09	-0.09	199-212		
					1953.12	1953.16	-0.04	65-81		
10	Trypsin	4 peaks match with histone H1c	Human	Q7658 (NCBI)	1327.75	1327.68	0.19	24-46	23	
					1579.70	1579.75	-0.01	65-78		
					1707.65	1707.89	-0.24	64-79		
					2147.17	2147.53	-0.36	1-21		
					1183.16	1183.38	0.10	230-239		
11	Trypsin	6 peaks match with ribosome S4	Human	P12750 (Swiss-Prot)	1216.38	1216.39	0.00	134-144	23	
					1354.03	1353.61	0.42	230-241		
					1567.81	1567.89	0.12	108-210		
					1557.75	1557.98	-0.23	37-48		
					2140.34	2140.58	-0.24	221-239		
					2591.80	2591.90	-0.10	77-98		

Figure 20. Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

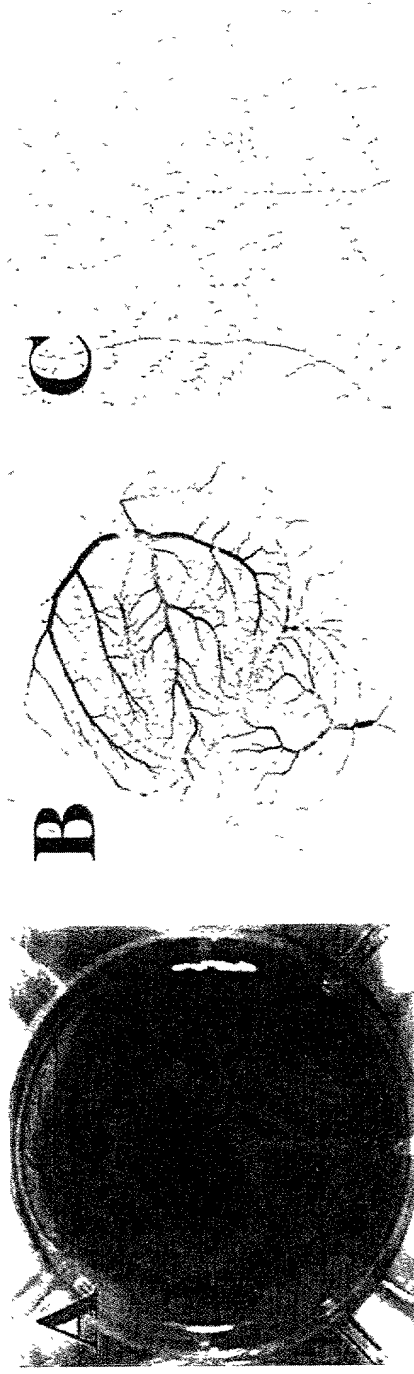


Figure 21. Black and white images of CAM vasculature after growth factor treatment

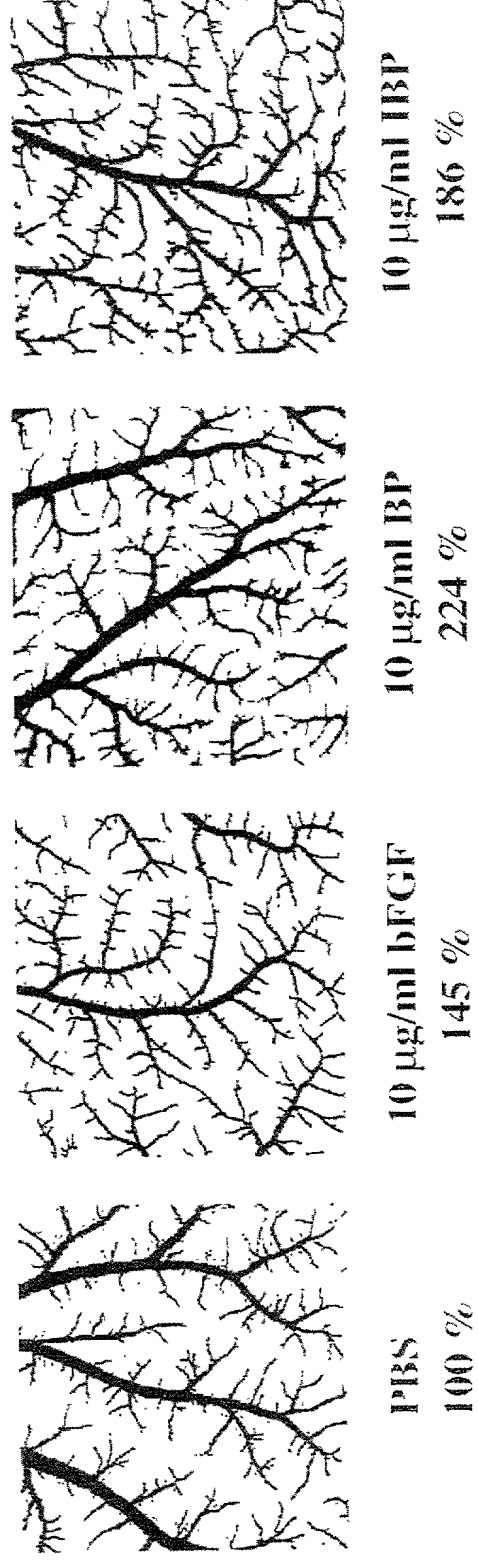
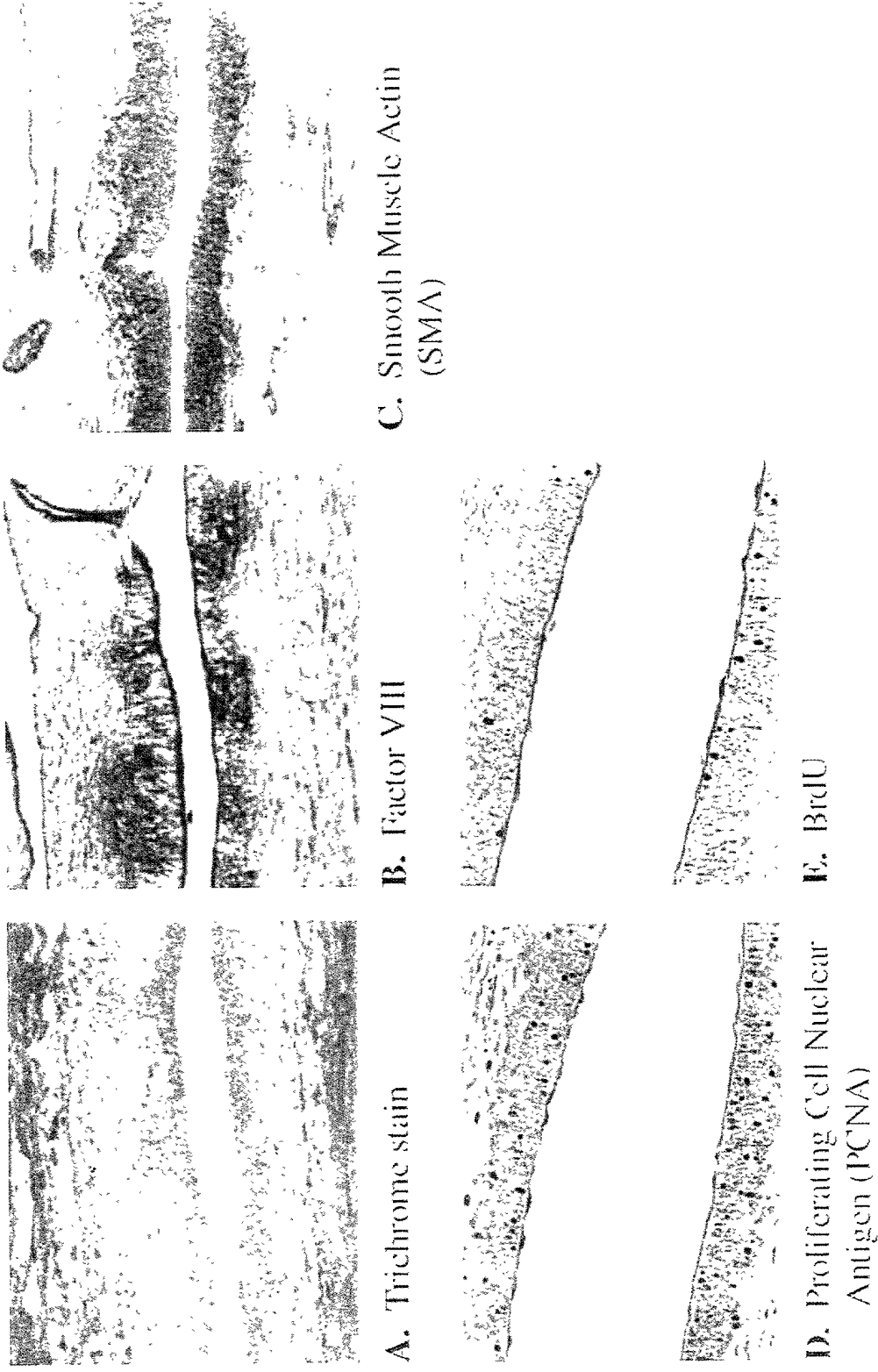


Figure 22. Histological sections of blood vessels formed in canine myocardium 2 weeks following BP injection



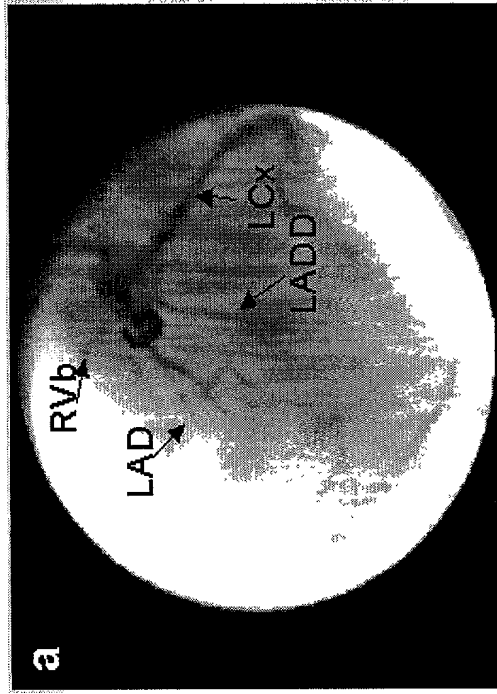


Fig. 23

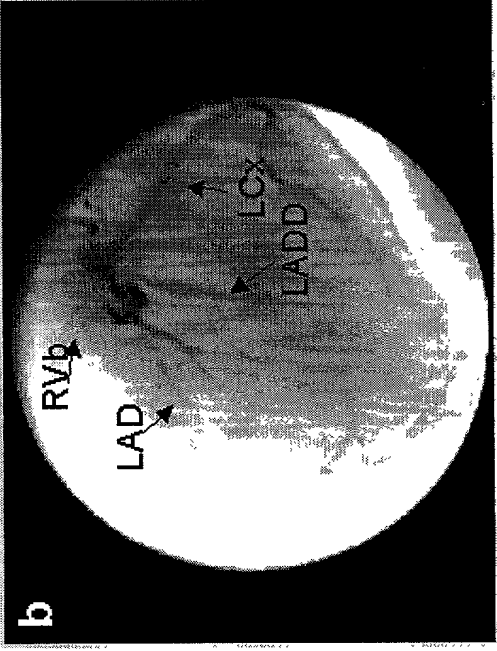


Fig. 24

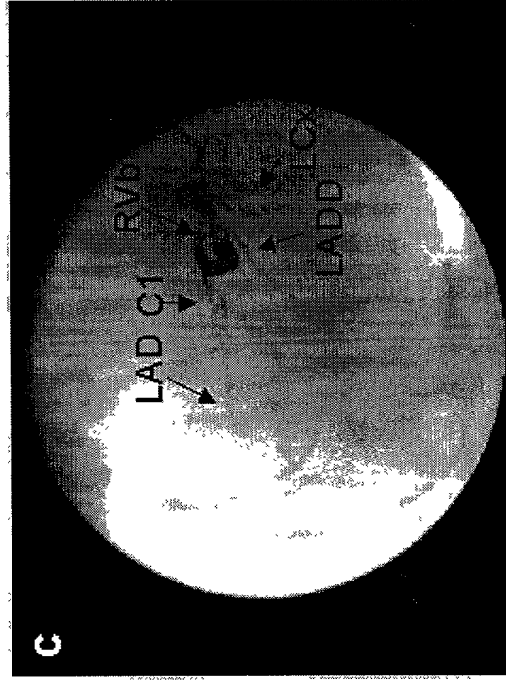


Fig. 25

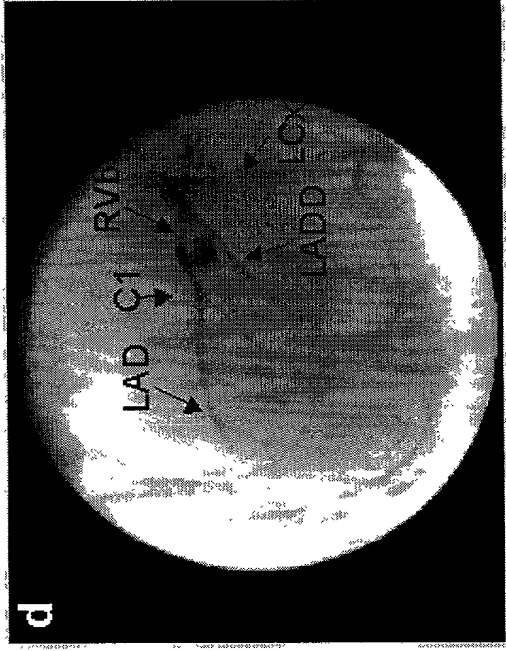


Fig. 26